CERIC OXIDATIONS OF AROMATIC STEROIDS AND RELATED COMPOUNDS.

by

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Summary

The ceric ammonium nitrate oxidation of a series of oestratriene derivatives was carried out, and the products were isolated and identified. The principal oxidation product of all the oestratrien compounds examined was the 9α-hydroxy-11β-nitrate compound. Several non-aromatic steroids were also oxidised by this ceric reagent. In only three cases, namely dihydrolanosteryl acetate, cholesterol, and cholest-5-enone were identifiable products obtained. All other non-aromatic steroids yielded only starting material and unidentifiable degradation products. A series of simple non-steroidal aromatic compounds was oxidised in the same manner and the products were identified.

An attempt was made to determine the mechanism of the ceric ammonium nitrate oxidation of oestrone acetate. The reaction was shown to involve at least two stages; the first being a radical oxidation at position 9; and the second a complex non-radical oxidation, leading to a high yield of the 9α-hydroxy-11β-nitrate ester derivative.

The failure of non-aromatic steroids to react cleanly with ceric ammonium nitrate showed the necessity for the presence of an aromatic ring with free benzylic hydrogens, or a conjugated olefinic bond. The presence of nitrate ion was also shown to be necessary for successful oxidation to occur.

Some of the reactions of the primary oxidation product of oestrone acetate, the 9α-hydroxy 11β-nitrate ester, were investigated. The 9α,11β-dihydroxy derivative was obtained by reduction from the nitrate ester and some of the reactions of this compound were also examined.

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INTRODUCTION

Introduction.

The only tetrapositive lanthanide which is sufficiently stable to exist in aqueous solution as well as in the solid state is Cerium. Cerium (IV) is obtained in practise by treating a cerium (III) solution with a strong oxidising agent such as peroxo-disulphate in nitric acid, or permanganate etc. It readily forms double salts, the best known being ceric ammonium nitrate, $Ce(NO_3)_4.2NH_4NO_3$. The aqueous chemistry of cerium (IV) is that of a strong oxidising agent and, until recently, it has been chiefly used as an analytical reagent. The highly charged Ce(IV) ion has a pronounced tendency to hydrate to yield $Ce(H_2O)_n^{4+}$, although it is probable that this particular species exists only in perchloric acid solutions. In other media, however co-ordination of anions doubtless occurs and this explains the variation in the potential of Ce(IV)/Ce(III) couple with the nature of the acid medium,[1]

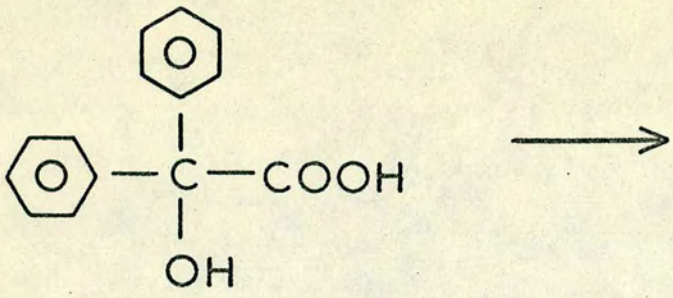
$$Ce(IV) + \epsilon \longrightarrow Ce(III), \quad \begin{matrix} Eo\ +1.38 \text{ in } 1N.HCl. \\ Eo\ +1.70 \text{ in } 1N\ HClO_4 \end{matrix}$$

Nevertheless, in the list of standard oxidation potentials, Ce(IV) is rated as one of the stronger oxidising agents, and consequently the reagent has been widely used to determine other inorganic species including antimony[2], arsenic[3], iodide[4], chromium[5] and ferrous iron[6].

The actual cerium (IV) species used include ceric sulphate, ceric perchlorate, and ceric ammonium nitrate*, with solvents such as sulphuric acid, perchloric acid, hydrochloric acid, nitric acid, and water.

In the field of organic chemistry, cerium (IV), also called ceric, has, until comparatively recently, been used mainly in the determination of simple organic acids and hydroxy compounds[7]. In general, ceric

* Hereafter referred to as CAN.



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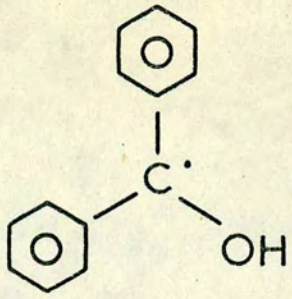

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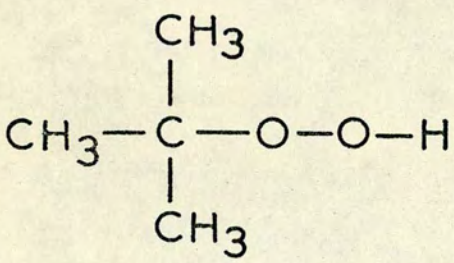

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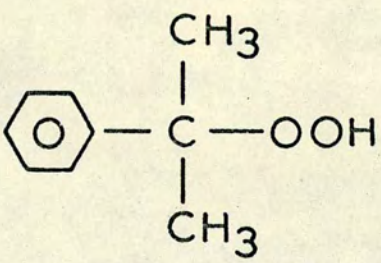

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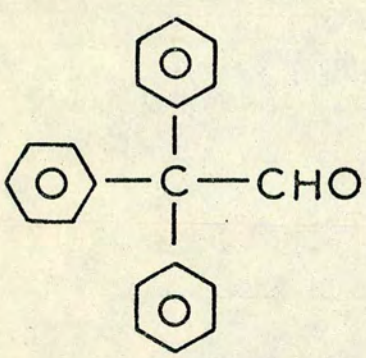

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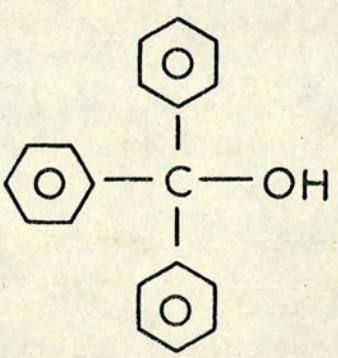

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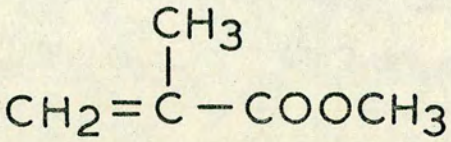

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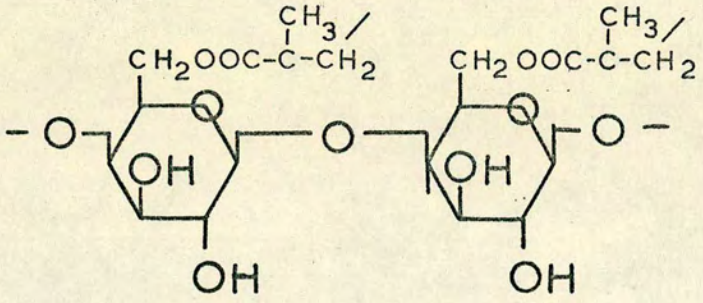
oxidations involve the complexing of a ceric ion to various ligands, and the precise nature of such a complex depends on the ceric species used, the acidic medium, and the compound being oxidised. Ligand complexing to ceric was shown to be important by Wiberg and Ford[8], who reported on the kinetics of oxidation of benzaldehyde to benzoic acid using ceric perchlorate, whereas Trahanovsky et al[9] found that, during the oxidation of benzyl alcohol to benzaldehyde with ceric sulphate, the aldehyde was not further oxidised, in acetic acid. In the presence of perchloric acid therefore, the oxidant is promoted to a level where it can oxidise the aldehyde to benzoic acid. The actual oxidative species is generally not known but recently Grover and Gupta[10] reported that the oxidation of benzilic acid[1] in sulphuric acid involves the oxidative species, $Ce(OH)_2^{2+}$, which complexes to the benzilate ion. This complex then decomposes to give a cerium (III) ion, carbon dioxide and a diphenyl methanol radical [2]. In this instance formation of a complex was shown by the appearance of a peak in the ultra violet spectrum at 255 nm, at which wavelength, both reagents were shown to be transparent. After complexing, the ceric ion abstracts an electron to give a radical. Attack by another ceric ion on the radical formed then yields the product, in this case, benzophenone.

Similar production of radicals by ceric oxidation has been shown by several methods. Oxidation of hydroperoxides yields peroxy radical intermediates, whose presence can be substantiated from their E.S.R. spectra. Such spectra have been obtained for t-butyl hydroperoxide [3], and cumyl hydroperoxide [4], with line width of 14 and 6 gauss respectively[11]. Radical intermediates have also been shown to be present by the polymerisation of acrylamide during the reaction between ceric and benzaldehyde. In the absence of benzaldehyde, less polymerisation occurred[12]. Similarly, during the oxidation of



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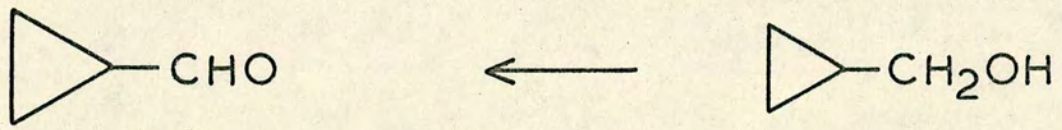

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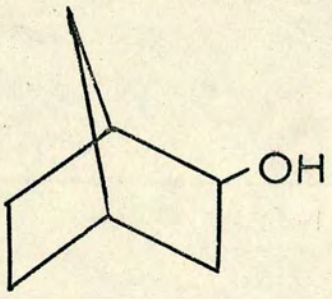

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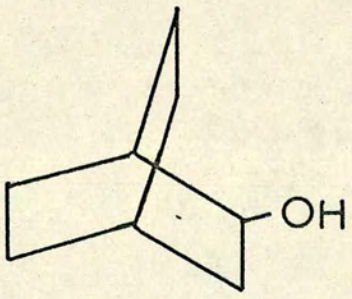

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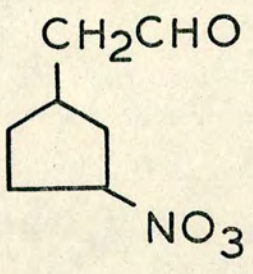

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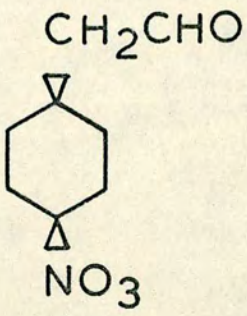

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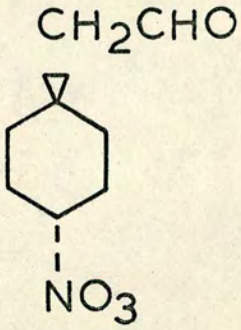


[15]

of triphenylacetaldehyde [5], triphenylcarbinol [6] was obtained in 75% yield. This reaction involves the formation of an acyl radical which decarbonylates to give a trityl radical and this is further oxidised to give triphenyl carbinol[12].

Ceric oxidations which produce radical intermediates show that cerium oxidises the substrate initially by a one-electron step reaction. This has been confirmed by detection of the unstable thallium (II) species in the oxidation of thallium (I) to thallium (III) by ceric nitrate[14], and the oxidation of chromium (III) to chromium (VI) has been shown to follow the same one-electron sequence[14]. These results are also compatible with the use of CAN to promote polymerisation in the formation of block co-polymers. Iwakura[15] has demonstrated that CAN can be used to graft methyl methacrylate [7] on to poly (6-methacryloyl D-galactose)[8]. This gave a block co-polymer product, containing branches with, on average, fifty methyl methacrylate units. Several other reactions which show that ceric reacts by a radical mechanism have been reported[16].

The stoichiometry of these oxidation reactions is never exact[17], but, in general, it would appear that two moles of ceric are consumed per mole of carbon-hydrogen bond broken[18].

In analysis, compounds to be determined by ceric are generally treated with an excess of ceric ion, and the excess is then back-titrated with a suitable reagent and indicator eg ferrous iron and ferroin[19]. Oxalic acid, however can be titrated directly with ceric sulphate or perchlorate[20]. Willard and Young[21], and, later, Sharma and Mehrota[22], have formulated the optimum conditions required for the analysis of some ten organic acids. Other compounds which can be readily determined by ceric oxidation include hydroxy compounds, (alcohols[18], hydroxy acids[18], and carbohydrates[23]) and reduced quinones[24].

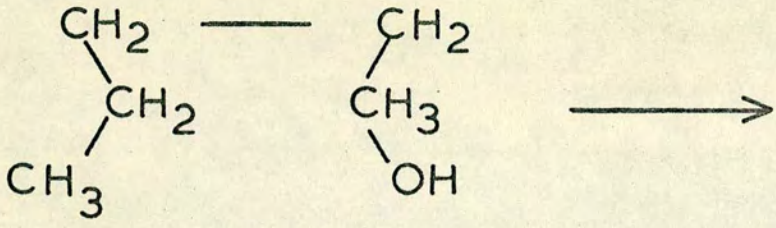
In recent years, interest has turned away from the purely analytical aspects of ceric oxidations to the reaction products themselves and ceric oxidation is now being widely used as an oxidative method permitting hitherto difficult reactions to be performed relatively easily[25].

The two most common ceric reagents used are ceric sulphate and CAN, although ceric perchlorate is also used. Ceric sulphate and CAN are most frequently employed since they remain stable in aqueous solutions. Ceric perchlorate is prepared by dissolution of ceric hydroxide in perchloric acid, but this reagent is unstable and slowly loses its oxidising properties on standing[26]. Reactions are most commonly carried out in acid solutions to ensure that the ceric remains in solution; should the pH rise, ceric precipitates as ceric hydroxide, or other more complex species, including polymeric species[27]. Ceric sulphate is less soluble than CAN, and this can cause some difficulties, particularly with regard to reactions with organic substrates. For example, it is found that ceric sulphate is only very sparingly soluble in 50% acetic acid, less than 0.5 g per 100 mls, but CAN is very soluble in this medium. CAN is also very soluble in water whereas ceric sulphate is only very sparingly soluble and solution is accompanied by decomposition.

Solution of CAN in water results in a large drop in pH due to the dissociation of the reagent to liberate nitric acid. X-ray diffraction studies[28] and theoretical prediction[29] both show that the ceric ion in solution is 8- co-ordinated, and this can explain the observed drop in pH[30],

<u>ie</u> $Ce(NH_4)_2(NO_3)_6 \xrightarrow{2H_2O} Ce(H)_2(NO_3)_6 + 2NH_4OH$

$Ce(H)_2(NO_3)_6 \xrightarrow{nH_2O} Ce(H)_2(OH)_n(NO_3)_{6-n} + nHNO_3$



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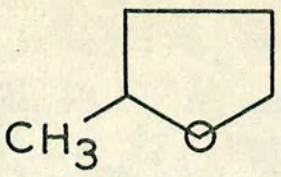

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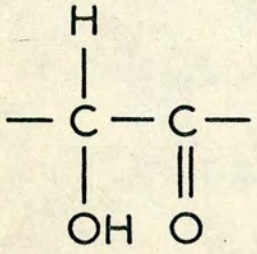

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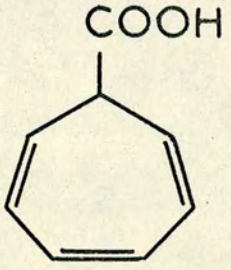

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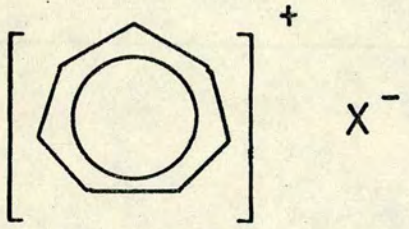

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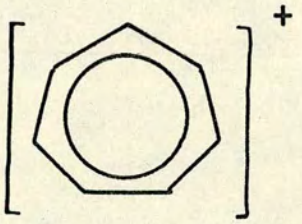

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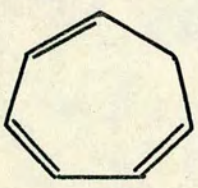

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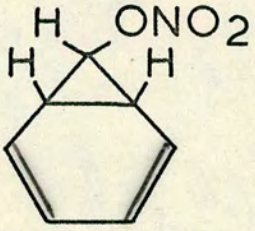

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The ceric ion has been shown to oxidise by forming a complex with the substrate, this being followed by the abstraction of an electron. Such complex formation is demonstrated by a red shift in the visible spectrum, when ceric is allowed to react with ethanol[31]. In this reaction, the electron abstraction is the rate-determining step. The ceric is complexed to the hydroxyl group and a carbon-hydrogen bond on the α-carbon atom breaks to give a radical species and a cerous ion. A further one electron oxidation of this radical yields the corresponding carbonium ion which upon further oxidation by ceric yields the observed reaction product, acetaldehyde, in 90% yield[32].

In general alcohols are oxidised to the corresponding aldehydes or ketones. Cyclopropanecarbaldehyde [9] is obtained in 64% yield from cyclopropanemethanol [10] by the action of CAN[25], whilst benzaldehyde can be obtained in 94% yield from benzyl alcohol[33].

Trahanovsky et al [34, 35] have recently reported two alternative reaction pathways operating in special cases. Bicycloheptanol [11] and bicyclooctanol [12] when reacted with two moles of CAN underwent ring-opening to yield unsaturated cyclic aldehydes in approximately 40% yield, whilst the major product was a nitrato-aldehyde, observed in about 50% yield. Bicyclo- 2,2,1 -2-heptanols [11] yielded 3-nitrato-cyclopentane-acetaldehyde [13], and bicyclo- 2,2,2 -2-octanol [12] yielded a mixture of cis- and trans-4-nitrato-cyclohexane acetaldehyde [14,15] respectively . This was the first recorded instance of ceric oxidation yielding a nitrato compound[34].

In 1967, Trahanovsky[35] reported that when n-pentanol [16] was reacted with two moles of CAN, it cyclised to yield 2-methyltetrahydrofuran [17], in 20% yield, as the only isolable product. No other example of this type of reaction has since been reported.

Ceric can also be used to oxidise glycols[18]. Again the reaction

proceeds by complex formation to one of the hydroxyl groups. The overall reaction usually results in cleavage to give a high yield of product; for example, pinacol is oxidised to acetone[36], and glycerol to formic acid[37]. This reaction is used in the quantitative determination of glycerol by ceric. In contrast to the oxidation of ethanol, the rate determining step in the glycol reaction was shown to be the rupture of the carbon- carbon bond between the carbon atoms bearing the two hydroxyl groups. This became apparent from a detailed study of the reaction between ceric sulphate and butane-2, 3-diol using isotopic substitution techniques[38]. These studies revealed no isotope effect in the reaction thereby indicating that hydrogen-oxygen bond-fission is not involved in the rate-determining step.

Aliphatic ketones can be oxidised by ceric sulphate and the reaction has a rate-determining step involving the breakage of the α-carbon-hydrogen bond. Here again ceric initiallycomplexes to the carbonyl double bond and this complex on decomposition yields a radical intermediate, further oxidation of which leads to α-ketol [18] formation[39,40]. Littler[39] has shown that the ketol [18] can be further oxidised by ceric to give an α-dione, which in turn reacts further to yield, in the case of cyclohexanone, adipic acid in approximately 60% yield[39]. Shorter[40] showed that acetone can be oxidised with CAN to a mixture of acetic acid and formic acid. Generally, high molecular weight ketones undergo extensive oxidative degradation, giving mainly mixtures of acetic and formic acids[12]. Indeed, the higher the molecular weight of the ketone, the more ceric is consumed and the more formic acid is formed. Results described in this thesis would indicate that the steroidal ketones such as the 17-carbonyl group of oestrone and the 3-carbonyl group of androstanolone are not readily oxidised by CAN. The reactions generally used short periods of time, up to three hours, and mild conditions,

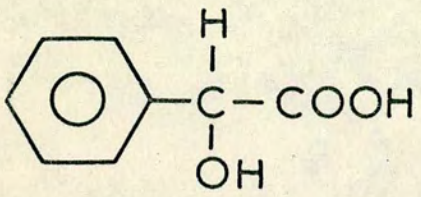
CAN in aqueous acetic acid, and in no case was an isolated carbonyl group found to be oxidised. Reaction for longer periods of time *eg* overnight, however resulted in extensive degradation of the steroid molecule. This degradation most probably occurred by initial reaction of the steroidal ketone.

Aliphatic aldehydes undergo similar reactions to aliphatic ketones, with rupture of the carbon-hydrogen bond on the carbon α to the carbonyl group[41].

The oxidation potential of Ce(IV) is so great that acids themselves can be oxidised. Formic, acetic, and propionic acids remain unoxidised by ceric sulphate, although acetic and formic acids are slowly oxidised by ceric perchlorate to carbon dioxide and water[42]. There are many examples of the oxidation of acids quoted in the literature[42]. In the special case of cyclohepta-2, 4, 6-trienecarboxylic acid [19], CAN oxidation results in the formation of tropenium salts [20][43]. This unusual reaction is doubtless founded upon the stability of the tropylium ion [21] due to its aromatic character. It has been reported by Trahanovsky[44] that the CAN oxidation of cycloheptatriene [22] yields benzaldehyde, benzene and carbon monoxide. This reaction is believed to occur via the tropylium ion which rearranges to give benzaldehyde via a norcaradiene intermediate [23].

It has been shown that light affects the stability of benzoic acid toward ceric sulphate[21,45], but no report has yet been made as regards the mechanism of this particular reaction.

There have been differing reports on the stability of dicarboxylic acids towards ceric oxidation. Malonic and oxalic acids are oxidised to carbon dioxide and water[22], but Willard and Young[21] reported that succinic, maleic, and fumaric acids were stable to ceric sulphate, whereas Sharma and Mehrota[22] using more vigorous conditions reported



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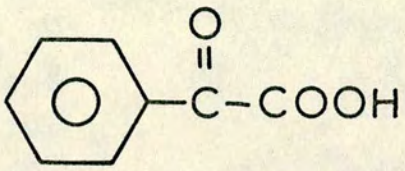

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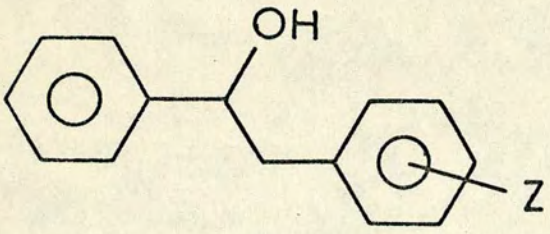

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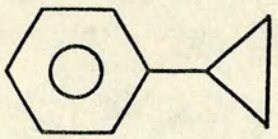

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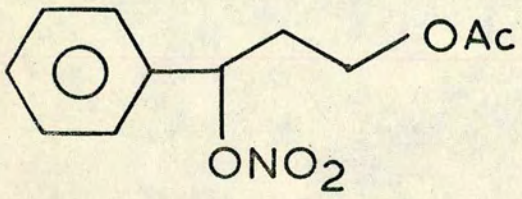

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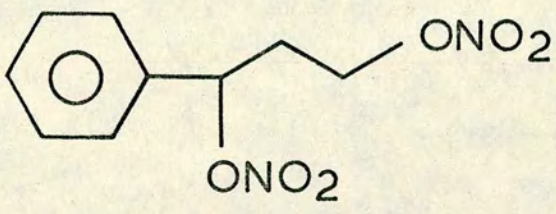


[29]

oxidation of these acids to acetic and formic acids.

Hydroxy acids are also oxidised by ceric compounds. Mandelic acid [24] is oxidised at the α-position to give the α-keto acid [25], but lactic and malic acids undergo more complex reactions to give, in the former case, 30% formic acid, and in the latter, 50% formic acid[17]. This reaction is used to determine lactic acid with ceric sulphate or ceric perchlorate as oxidant.

Bird and Diaper[46] have reported the conversion of oximes to the parent carbonyl compounds by the action of CAN. Yields varied from 90% for acetophenone to about 30% for camphor.

Little work on the ceric oxidation of hydrocarbons had been reported until a few years ago, but, during the last six years, there has been increasing interest in these oxidations, mainly due to the work of Trahanovsky. The study of hydrocarbon oxidation has been almost totally directed toward benzene derivatives.

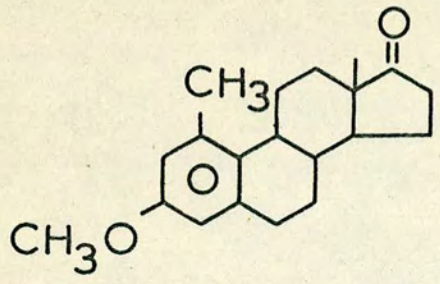
In 1966, Syper[47] reported that CAN could be used to oxidise toluene and substituted toluenes to the corresponding benzaldehydes. He reported yields, varying from 60% to 100% over a sample of some twenty substituted toluenes ranging from toluene itself to tetralin. The following year, Murti and Pati[48] published details of the kinetics and mechanism of this reaction and showed that the reaction had a rate-determining step involving the formation of a benzyl radical. They also reported that deactivating groups substituted on the aromatic ring retarded the reaction. Trahanovsky[9] has reported the use of Ce(IV) to oxidise variously substituted benzyl alcohols to their corresponding aldehydes. With CAN as oxidant, no further oxidation occurred because of the absence of labile α-hydrogens. Yields of over 90% were obtained for several substituted benzaldehydes, though more complex substitution usually resulted in lower yields. In the same

year that Syper[47] published on the oxidation of toluene, Trahanovsky and Young[49] reported results for the same reaction. These results differed somewhat from the results of Syper whose reaction yields were rather higher. This discrepancy can be explained by the fact that Syper used more vigorous conditions (ie stronger acid) than did Trahanovsky.

In 1970, Dust and Gill[50] studied the same reaction and found a high yield of 2-methyl benzyl nitrate was obtained when the oxidation of o-xylene was carried out in dilute acid. The yield of nitrate compound could be increased by using an aliphatic hydrocarbon solvent. They also said that toluene gave benzyl nitrate in good yield.

Trahanovsky and Young[49] also reported that, with anhydrous acetic acid as solvent for the oxidation, high yields of the corresponding benzyl acetates were obtained. Deactivating groups in the aromatic ring resulted in a drastic reduction in yields of the aldehydes obtained. This suggested that the deactivating group hindered the formation of the initial complex or the formation of the radical intermediate, results which were in agreement with those of Murti and Pati[48]. Similar substitution effects are reflected in the relative reaction rates reported for the oxidative cleavage by Ce(IV) of several substituted 2-aryl-1-phenyl ethanols [26][51]. These results indicated that the para-methyl compound reacted much faster than the para-chloro compound and this, much faster than the para-nitro. These results are in agreement with the deactivating effects of substituents on aromatic rings towards electrophilic substitution. It was also noted by Nave[51] that compounds substituted with an alkyloxy group were oxidised very much faster.

In 1968, Young[52] reported on the oxidation of arylcyclopropanes [27] using CAN. He isolated nitrate [28] and di-nitrate esters [29] in yields



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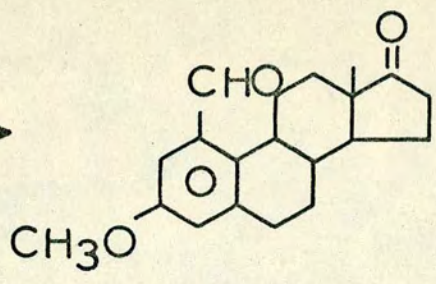

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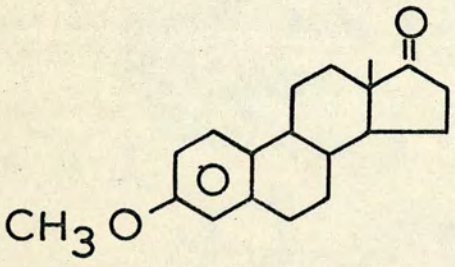

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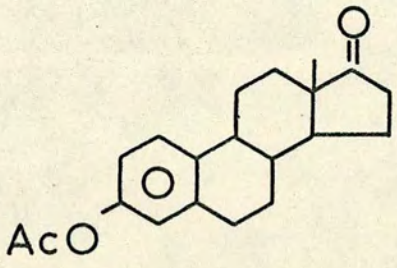

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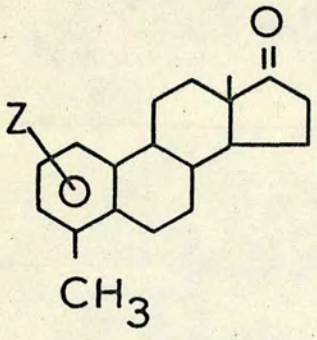

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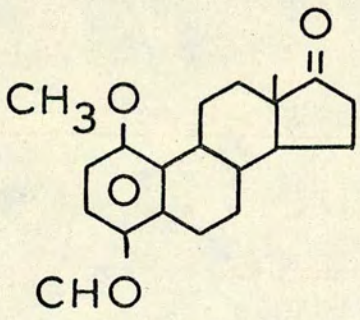

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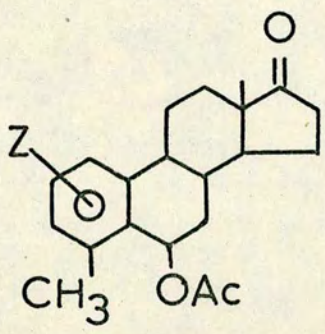


[36]

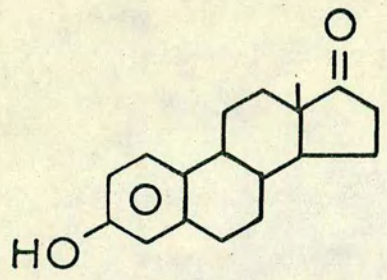
of up to 50% and concluded that carbon-cerium bonds were not formed during the reaction. This suggested initial complexing of the Ce(IV) to the aromatic ring had occurred and this had been followed by electron abstraction.

No work had been done on the oxidation of steroids with ceric, prior to 1968, when Laing and Sykes[53] reported that CAN oxidation of 3-methoxy-1-methyl oestra- 1, 3, 5(10)-trien-17-one [30] resulted in the formyl analogue [31]. The resultant 1-aldehyde was then decarbonylated using tris (triphenylphosphine) rhodium chloride to give 3-methoxy oestrone [32]. When however the reaction between CAN and 3-hydroxy oestra-1, 3, 5(10)-trien-17-one 3-acetate (oestrone acetate) [33] in aqueous acetic acid was studied, the reaction gave, in 70% yield, a product which had the infra-red spectrum of a nitrate ester but was otherwise of unknown constitution[54].

Subsequent to the completion of the work in this thesis, Piatak and Eichmeier[55] have reported that CAN oxidation of substituted 4-methyloestra-1, 3, 5(10)-trienes [34] only yielded a 4-aldehyde [35] when the compound contained a C-1 methoxyl group. /This showed that the position of Other substituents yielded a 6β-acetate [36].
benzylic oxidation, which occurred on CAN oxidation was affected by ring substituents.

In the light of the oxidations of aromatic hydrocarbons reported by other workers, it was decided to investigate the ceric oxidations of aromatic steroids (oestrogen) in detail.

DISCUSSION



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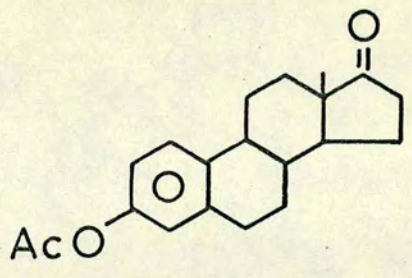

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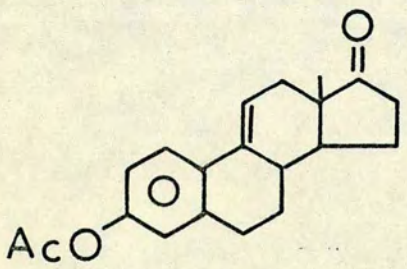

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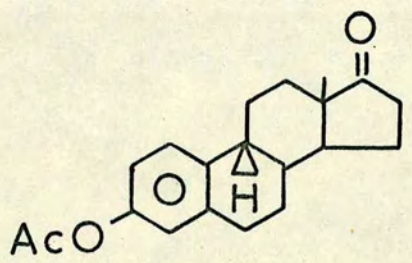

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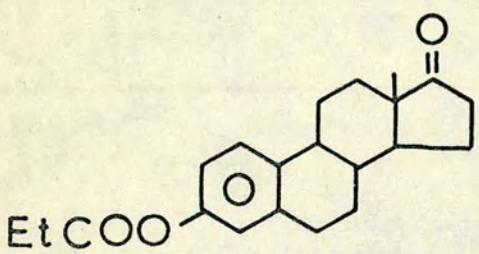

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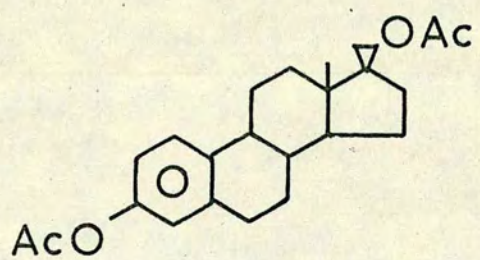

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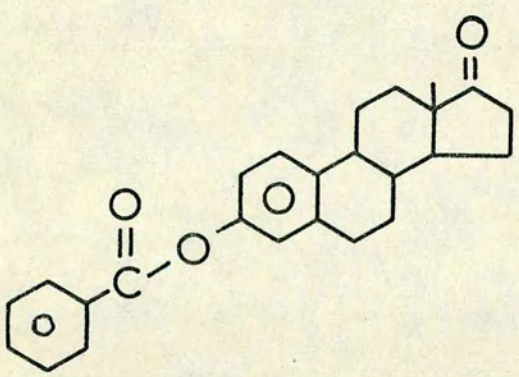

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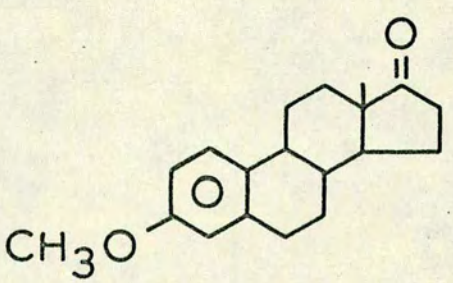


[44]

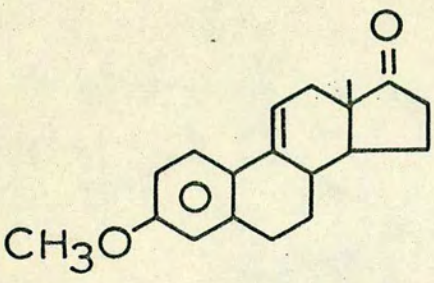
Preparation of Starting Materials.

Oestrone derivatives.

The following derivatives of oestrone [37] were prepared using standard methods as described in the experimental section.

Compound	Structure	Lit MP	Ref
3-Hydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (oestrone acetate)	38	123-4°	56
3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate (Δ9(11)-oestrone acetate)	39	128-9°	57
3-Hydroxy-9β-oestra-1,3,5(10)-trien-17-one 3-acetate	40	-	-
3-Hydroxyoestra-1,3,5(10)-trien-17-one 3-propionate (oestrone propionate)	41	134-6°	58
3,17β-Dihydroxyoestra-1,3,5(10)-trien 3,17β-diacetate (oestradiol diacetate)	42	125-6°	56
3-Hydroxyoestra-1,3,5(10)-trien-17-one 3-benzoate (oestrone benzoate)	43	217-5°	56
3-Methoxyoestra-1,3,5(10)-trien-17-one (oestrone 3-methyl ether)	44	167.5-9.5°	56
3-Methoxyoestra-1,3,5(10),9(11)-tetraen-17-one (Δ9(11)-oestrone 3-methyl ether)	45	142-5°	59
3-Benzyloxyoestra-1,3,5(10)-trien-17-one (oestrone 3-benzyl ether)	46	129-30°	60

All compounds exhibited the literature melting point.



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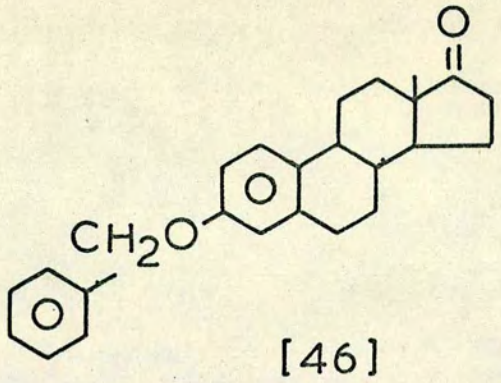

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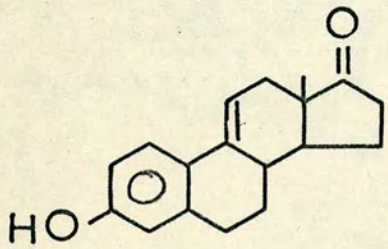

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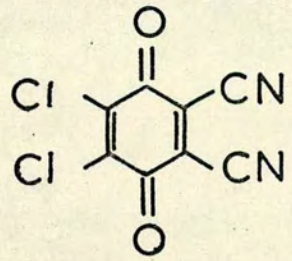

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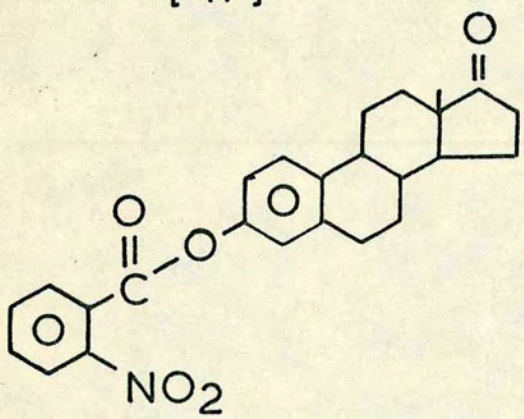

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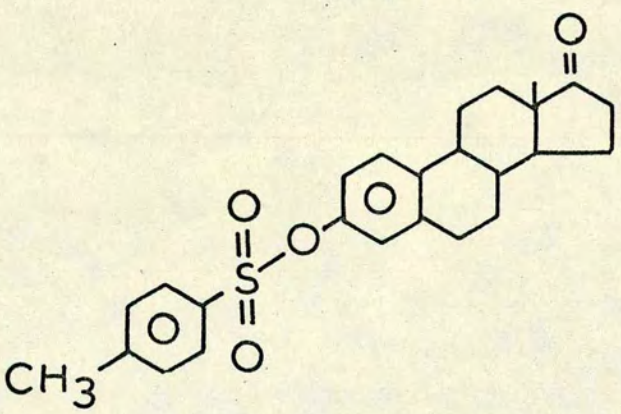

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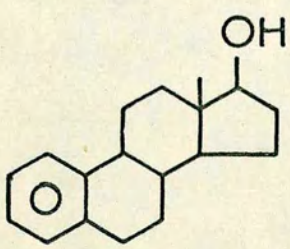

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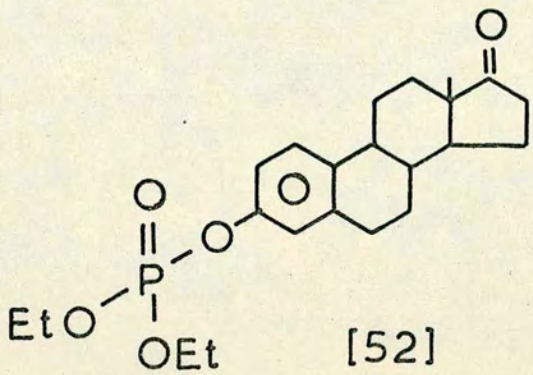


[52]

3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate. [39]

The compound was prepared by acetylation (with acetic anhydride/pyridine) of 3-hydroxoestra-1,3,5(10),9(11)-tetraen-17-one [47], which is the product resulting from the reaction of oestrone and dichlorodicyanobenzyoquinone (DDQ), [48][61]. The acetylation reaction gave a dark red crystalline product which, upon refluxing with decolourising carbon, gave white crystals of the 3-acetate, M.P. 125-7° (lit[57] MP 128-9°).

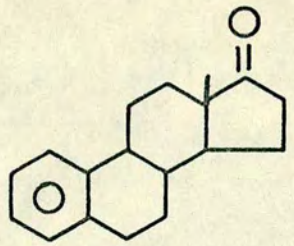
The mass spectrum of the impure acetate showed that the red contamination was due to the formation of a complex (of unknown constitution) between pyridine and DDQ. This was shown by the appearance of peaks for ions of mass, 79 (pyridine), and mass 236, 238, and 240 (DDQ), in addition to the parent ion peak of the steroid, mass 310. This contaminant in no way affected the reaction between the steroid and CAN.

Oestrone Esters.

Oestrone o-nitrobenzoate [49] and oestrone tosylate [50] were prepared by the standard reaction between oestrone and the corresponding acid chloride in pyridine, overnight at room temperature. Both compounds, previously unreported, gave correct analysis figures, and the spectral data were consistent with those expected for the compounds.

17β-Hydroxyoestra-1,3,5(10)-triene. (3-Desoxyoestradiol) [51].[62,63]

Attempts to prepare the diethyl phosphate ester of oestrone using diethyl chlorophosphite[62] as the esterifying agent proved unsuccessful. Solvents used for this reaction were an aqueous ethanolic solution of sodium hydroxide, and pyridine; in both cases less than 5% yield of the required phosphate ester was obtained. The phosphate ester was however prepared in good yield by the action of diethyl phosphite, triethylamine,



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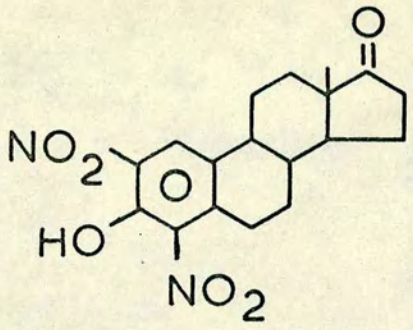

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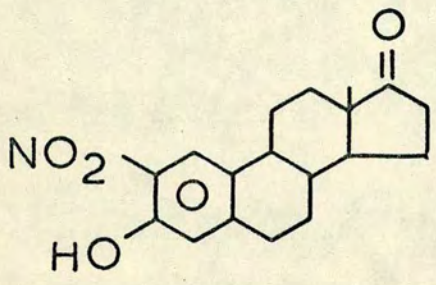

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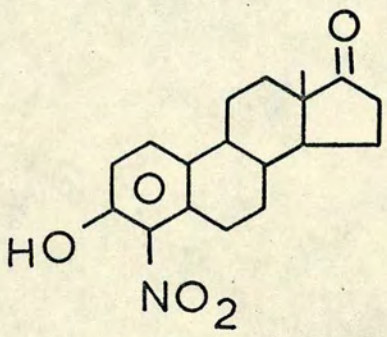

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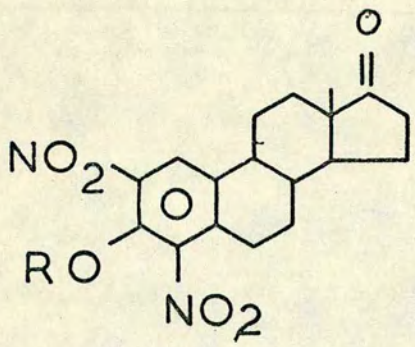
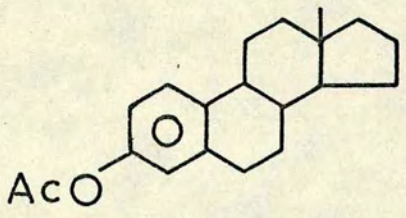


[57] a: R = Ac
b: R = CH_3



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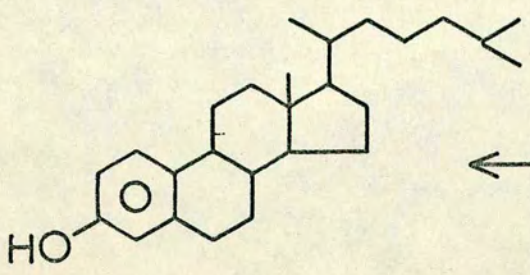

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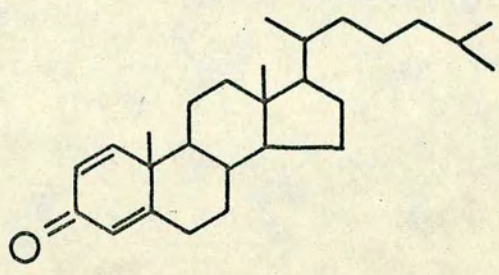


[60]

and carbon tetrachloride on oestrone in THF, as described by Caspi[63].

The diethyl phosphite, triethylamine and carbon tetrachloride reacted to give diethyl chlorophosphite in situ, which reacted with the oestrone in a normal acid chloride esterification reaction to give diethyl oestra-1,3,5(10)-trien-17-one-3-yl phosphate [52][63]. Treatment of an ethereal solution of the phosphate ester with lithium/liquid ammonia resulted in hydrogenolysis of the phosphate group to yield 3-desoxyoestradiol, M.P. 108-10° (lit[63] MP 109-10°).

Oestra-1,3,5(10)-trien-17-one (3-Desoxyoestrone) [53].[63,64]

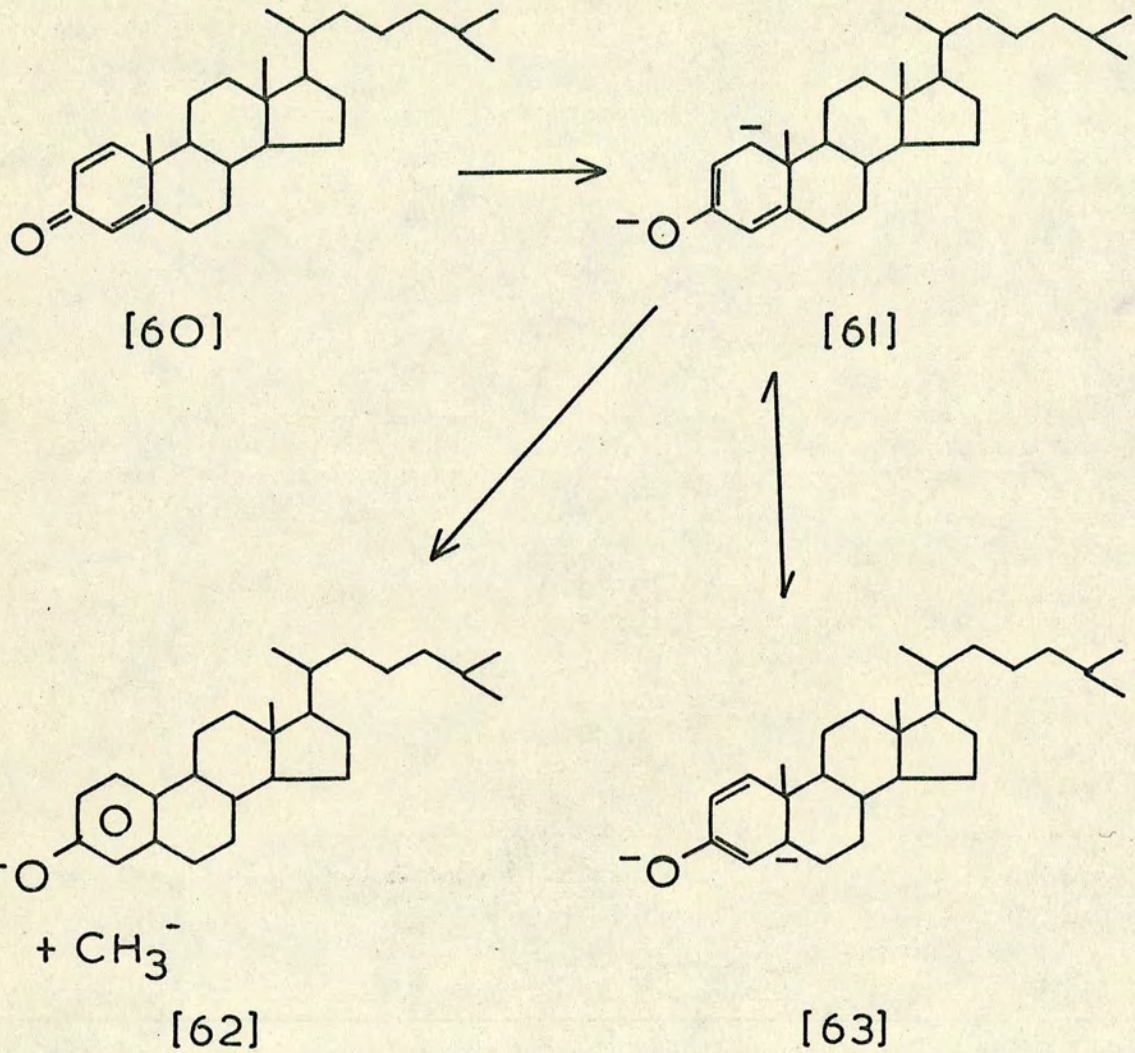
Oxidation of 3-desoxyoestradiol with chromium trioxide in pyridine[63] or with 8N chromic acid (Jones Reagent)[64] resulted in the formation of 3-desoxyoestrone, M.P. 149-1° (lit[63] M.P. 135-6°)

2,4-Dinitrooestrone [54][65,66]

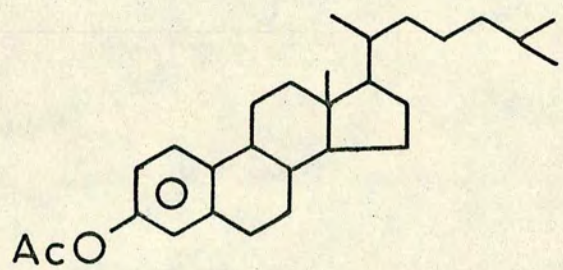
The nitration of oestrone was carried out according to the procedure of Niederl and Vogel[65], in an attempt to prepare 2-nitro-and 4-nitrooestrone [55,56]. However only one product was obtained, and this was identified as 2,4-dinitrooestrone, M.P. 186-7° (lit[66] M.P. 187-85°). I.R. and N.M.R. spectra, and T.L.C. all showed that no mono-nitro compound had been formed in the reaction.

2,4-Dinitrooestrone Derivatives.[66,67]

The normal acetylation procedure with acetic anhydride/pyridine yielded 2,4-dinitrooestrone acetate [57a], M.P. 186-8° (lit[66] M.P. 187-8.5°), mixed M.P. with dinitrooestrone 150-60°. The structure was confirmed by the N.M.R. spectrum. Treatment of 2,4-dinitrooestrone with an ethereal solution of diazomethane, prepared from p-toluene sulphonylmethyl nitroso amide in the usual manner[67], gave the previously unreported 3-methoxy-2,4-dinitrooestrone, [57b] M.P. 119-21°, confirmed by I.R. and N.M.R. spectra, and analysis.



[60] [61] [62] [63]



[64]

3-Hydroxyoestra-1,3,5(10)trien 3-Acetate. (17-Desoxyoestrone Acetate). [58][68]

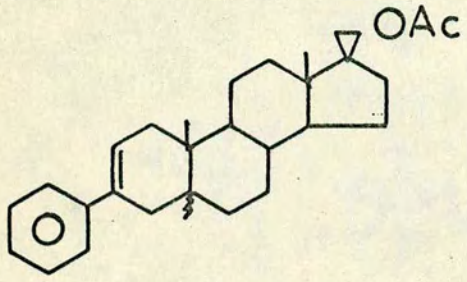
17-Desoxyoestrone was prepared by the Huang-Minlon[68] reduction of oestrone. The reaction was carried out using potassium hydroxide, and 85% hydrazine hydrate as reagents, with digol (diethylene glycol) as solvent. The 17-desoxy steroid was recrystallised from methanol and had a M.P. 138-41° (lit [68] M.P. 134-4.5°). The 3-acetate was prepared by treating the parent steroid with acetic anhydride/pyridine to give the previously unreported 3-hydroxyoestra-1,3,5(10)-triene 3-acetate, M.P. 84-6°, confirmed by I.R. and N.M.R. spectra, and analysis.

3-Hydroxy-19-norcholestra-1,3,5(10)-triene [59].[69]

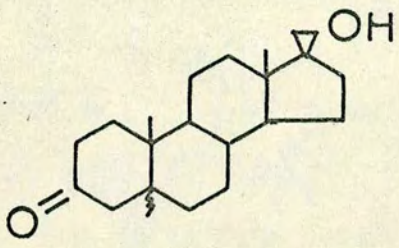
The aromatisation of cholesta-1,4-dien-3-one [60] to produce the title compound was carried out using lithium/biphenyl as reagent in THF as solvent[69]. The lithium and biphenyl reacted to give a radical anion, in great excess, which reacted with the dienone to aromatise the A-ring, with expulsion of the angular methyl group as methyl lithium.

The reaction is believed to occur via the intermediates, [61 - 63]. The dianion [61] stabilises itself by elimination of methyl carbanion with concurrent formation of a phenoxide ion [62]. The methyl carbanion elimination is probably aided by the presence of, and co-ordination with, lithium ion to give methyl lithium. Intermediate [63] exists in equilibrium with [61] and does not itself aromatise.

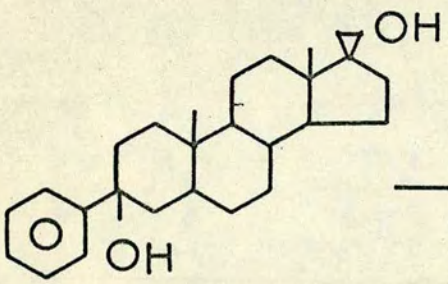
The 3-hydroxy-19-norcholesta-1,3,5(10)-triene had a M.P. 112-4° (lit [69] M.P. 118°) but was only prepared in 14% yield (reported [69] at 40%). The 3-hydroxy steroid was acetylated in the usual manner to yield the 3-acetate, [64], M.P. 91-3° (lit [70] M.P. 93.5-95°)



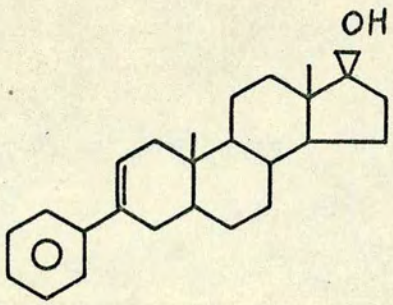
[65]



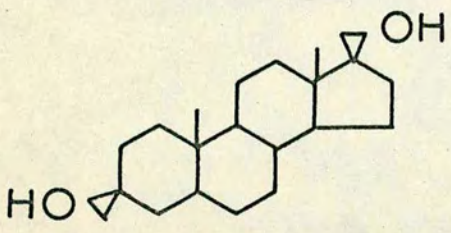
[66]



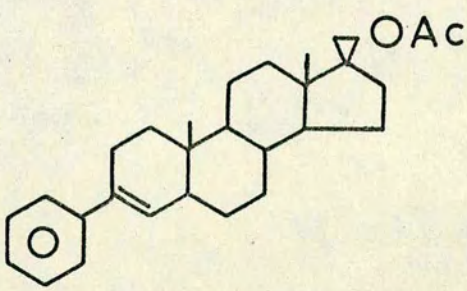
[67]



[68]



[69]



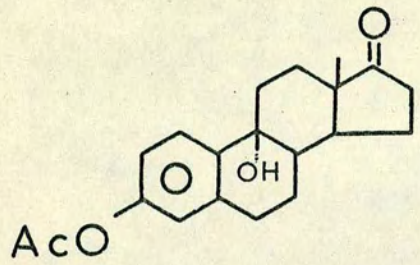
[70]

3-Phenylandrost-2-en-17β-ol 17β-Acetate. [65]

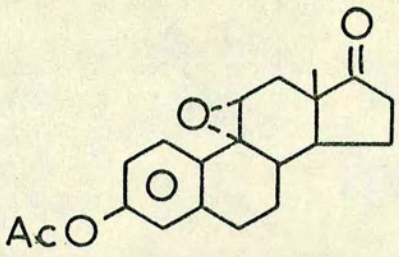
5α- Androstanolone [66] was treated with an excess of phenyl magnesium bromide[71] to give 3-phenylandrostan-3, 17β-diol [67]. T.L.C. showed some of this product had spontaneously dehydrated to give the required olefin, 3-phenyl-5α-androst-2-en-17β-ol [68]. Both compounds gave the same colour of spot on sulphuric acid development of the T.L.C. plate. This was presumed to be due to the 3-hydroxy compound [67] dehydrating during development to give the 2-olefin [68].

Acid dehydration with formic acid converted all the Grignard product to the olefinic steroid, still however contaminated by starting material and biphenyl, (a by-product of the Grignard reaction)[72]. Chromatography on a silica gel column enabled the biphenyl to be removed but did not separate the steroids, [66,68]. Sodium borohydride reduction converted the residual starting material to 5α-androstan-3β,17β-diol [69]. Further chromatography on an alumina column enabled the isolation of the required olefin.

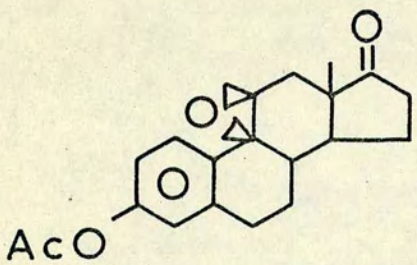
Acetylation in the usual manner gave the title compound [65], the N.M.R. spectrum of which showed that the product consisted of a mixture of two compounds, 3-phenyl-5α-androst-2-en-17β-ol 17β-acetate [65], 85%, and 3-phenyl-5α-androst-3-en-17β-ol 17β-acetate, [70], 15%, which could not be further separated.



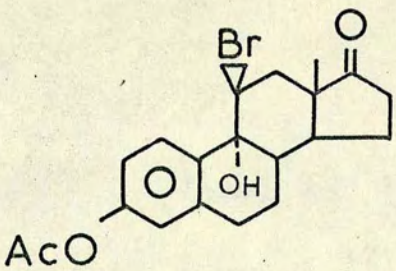
[71]



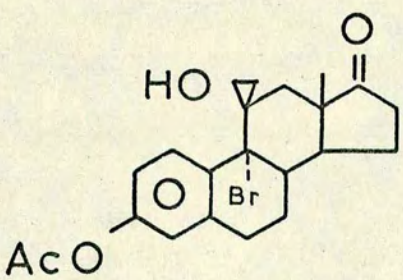
[72]



[73]



[74]



[75]

Preparation of Compounds for Investigation of the Mechanism of the Oestrone Acetate/CAN Reaction.

Several derivatives of oestrone acetate were suggested (see later in discussion) as possible intermediates in the CAN oxidation reaction. These were 3-hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate [39], the preparation of which has already been discussed (see P12), 3,9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate [71], 3-hydroxy-9α,11-epoxyoestra-1,3,5(10)-trien-17-one 3-acetate [72], and 3-hydroxy-9β, 11-epoxyoestra-1,3,5(10)-trien-17-one 3-acetate [73].

3,9α-Dihydroxyoestra-1,3,5(10)trien-17-one 3-acetate [71].[73,74]

3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate [39] was reacted with N-bromosuccinimide and perchloric acid for thirty minutes at 0°C[73] to give 11β-bromo-3,9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate [74]. Debromination with Raney nickel, prepared as in Organic Syntheses[74], gave 3,9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate [71], M.P. 166-8° (lit[73] M.P. 167-8°).

3-Hydroxy-9α,11-epoxyoestra-1,3,5(10)-trien-17-one 3-acetate [72].[73]

3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate [39] was epoxidised by reaction with m-chloro perbenzoic acid to give the 9α,11-epoxide [72], M.P. 160-2° (lit[73] M.P. 160-2°). None of the 9β,11-isomer [73] was observed in the crude or purified products.

Attempted Preparation of 3-Hydroxy-9β,11-epoxyoestra-1,3,5(10)-trien-17-one 3-Acetate [73].[75]

3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate was reacted with N-bromosuccinimide and perchloric acid for three hours at 0°C[75] to give 11β-bromo-3,9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate [74]. Dehydrobromination by refluxing with potassium acetate yielded 3-hydroxy-9α,11-epoxyoestra-1,3,5(10)-trien-17-one 3-acetate [72], M.P. 160-2° (lit[73] M.P. 160-2°). A previous account

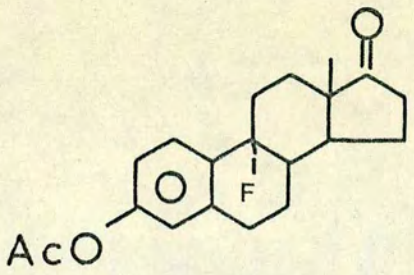
of this reaction[75] listed the initial product of the N-bromosuccinimide reaction as being 9α-bromo-3,11β-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate [75] , which, on dehydrobromination with potassium acetate, should result in the formation of the 9β,11-oxide [73], reported to have M.P. 149-51°[73]. Any 9β,11-oxide present as contaminant in the prepared compound, the 9α,11-epoxide [72], would be expected to lower the M.P. of the product. Formation of the 9β,11-epoxide would result in a large change in the molecular shape, due to inversion at C-9. This same inversion has been reported[76] to cause a downfield shift of the C-18-methyl signal in all cases examined. Examination of the N.M.R. spectrum of the product showed the C-18-methyl signal to occur at exactly the same frequency, 9.10τ, as the known 9α,11-epoxide. The product M.P. and N.M.R. spectrum taken together showed that no 9β,11-epoxide had been formed.

An alternative route by the attempted epimerisation of the 9α,11-epoxide by refluxing with potassium acetate in acetone resulted in a product which consisted only of starting material.

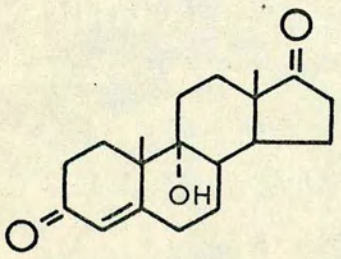
Attempted Preparation of 9α-Fluorooestrone Acetate [76].[77]

The product of CAN oxidation of oestrone acetate considered in conjunction with the known method of reaction of ceric suggested the initial step in the oxidation involved C-9 of the steroid. It was decided to react an oestrone derivative with C-9 bearing fluorine on the assumption that reaction would occur at the other benzylic carbon, C-6.

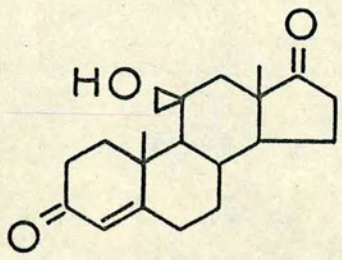
Several attempts to prepare 9α-fluorooestrone acetate [76] proved unsuccessful. In no case was an isolable yield of the required compound obtained. One routeattempted used the action of anhydrous liquid hydrogen fluoride on Δ9(11)-oestrone acetate with pyridine or methylene chloride as solvent. This was attempted on the assumption that the hydrogen fluoride might add across the double bond in a simple Marcownikow reaction.



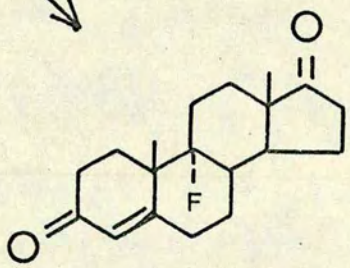
[76]



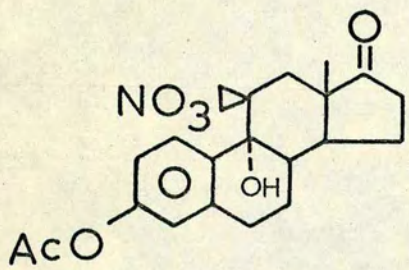
[77]



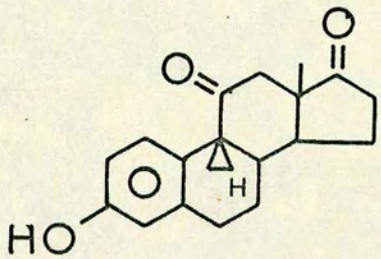
[78]



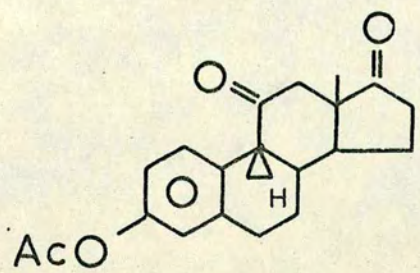
[79]



[116]



[149]



[124]

The only product obtained was hydrolysed starting material. Mass spectrometry confirmed that no fluorine had been incorporated into the steroid. Another route involved the reaction of 9α-hydroxyoestrone acetate with anhydrous liquid hydrogen fluoride in pyridine or methylene chloride as solvent. This was attempted because it has been reported that the action of hydrogen fluoride in pyridine on 9α-hydroxy-,[77], or 11β-hydroxyandrost-4-ene-3,17-dione[78] resulted in formation of 9α-fluoroandrost-4-ene-3,17-dione[79], although no yield was reported. With pyridine as solvent, the reaction of 9α-hydroxyoestrone acetate gave a product, which, from N.M.R. and mass spectrometry, was shown to consist only of the 9(11)-olefin, with a low return of unreacted starting material.

With methylene chloride as solvent, the reaction product was shown by mass spectrometry to have incorporated some fluorine, but the N.M.R. spectrum indicated that the required 9α-fluorooestrone acetate was present in less than 5% yield. In this reaction, as with the 9(11)-olefin, the primary reaction product was the free phenolic steroid, which was re-acetylated in the usual manner to improve the solubility in deuterochloroform to enable the running of the N.M.R. spectrum.

3-Hydroxy-9β-oestra-1,3,5(10)-triene-11,17-dione [149].

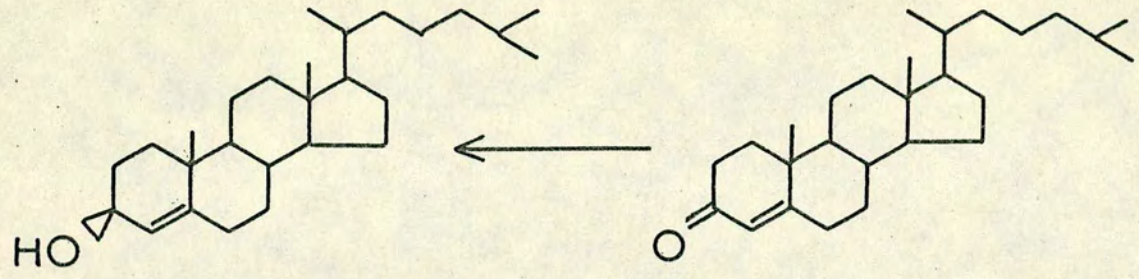
Subsequent upon the failure to prepare 9α-fluorooestrone, it was decided to prepare an oestrone derivative with C-11 blocked; this being the other position involved in CAN oxidation. If CAN could convert this to a 9-hydroxy compound this would confirm reaction at C-9 was an important, and probably primary, step.

Tsuda[73] has reported the conversion by strong base of the 9,11-bromohydrin [74] to the title compound [149] . Work detailed later in this thesis showed that the hydroxy-nitrate [116] formed by the reaction of oestrone acetate with CAN could also be used as starting material

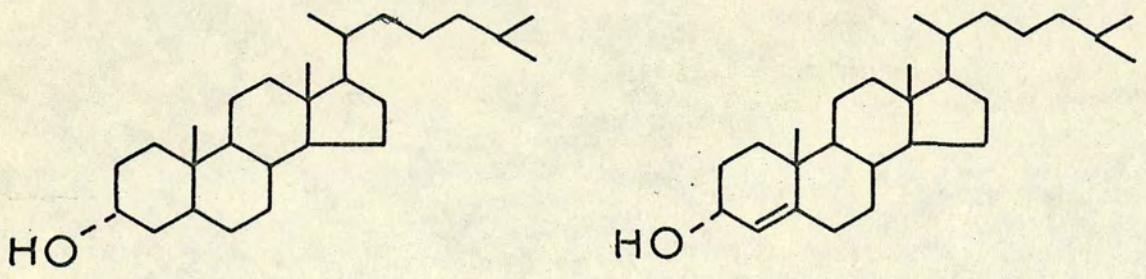
to prepare the title compound, and therefore this route was used.

The mechanism of action of strong base on the hydroxy nitrate, which results in the title compound is detailed later P58.

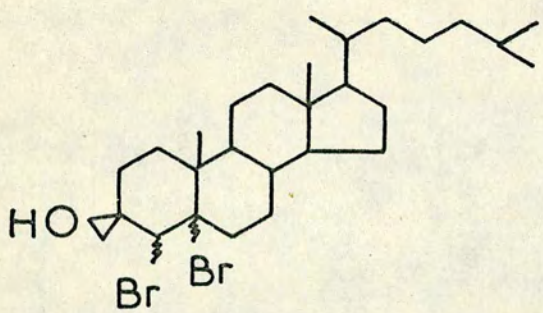
Acetylation in the usual manner yielded the acetate [124] of the title compound for reaction with CAN.



[80] [81]



[82] [83]



[84]

Non-Aromatic Steroids.

Cholest-4-en-3β-ol (Allo-cholesterol)[80].[78,79,80]

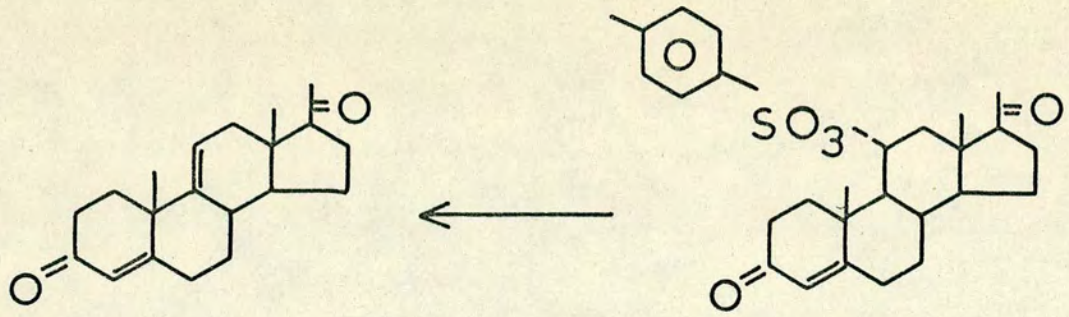
Allo-cholesterol[80] was prepared by two methods of reduction of cholest-4-en-3-one[81]. Lithium aluminium hydride reduction gave a mixture of the required product, and cholestan-3α-ol[82], which were separated by alumina chromatography. Reduction by lithium aluminium tri-t-butoxy hydride[78], prepared by the addition of the t-butanol to a suspension of lithium aluminium hydride in THF resulted in a mixture of the required product, and cholest-4-en-3α-ol[83], which were separated by crystallisation from acetone.

It was found that the standard method of purification of cholesterol, by bromination[79], could also be applied to the purification of allo-cholesterol. Addition of one mole of the bromine gave 4,5-dibromocholestan-3β-ol[84]; this compound was unstable to alumina chromatography undergoing extensive degradation. Chromatography onsilica gel could however be used to purify the compound if necessary. Debromination of the dibromosteroid[84] with zinc/ether in the usual manner[79] gave the required product, allocholesterol, in 75% yield, based on the allocholesterol content of the impure starting material. The compound had M.P. 132-3° (lit[80] M.P. 132°).

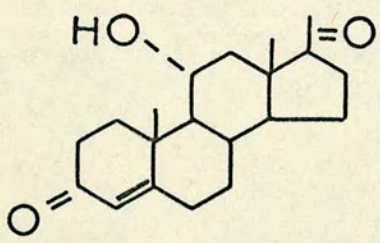
By analogy with the purification of cholesterol, the dibromo compound prepared above would be expected to add the bromines in a trans diaxial manner and therefore the compound would be 4β,5α-dibromo-allocholesterol. The compound was not characterised completely however. It was felt since the compound was only a purification intermediate, total characterisation was unnecessary.

Pregna-4,9(11)-diene-3,20-dione (Δ9(11)-progesterone)[85].[81,82,83]

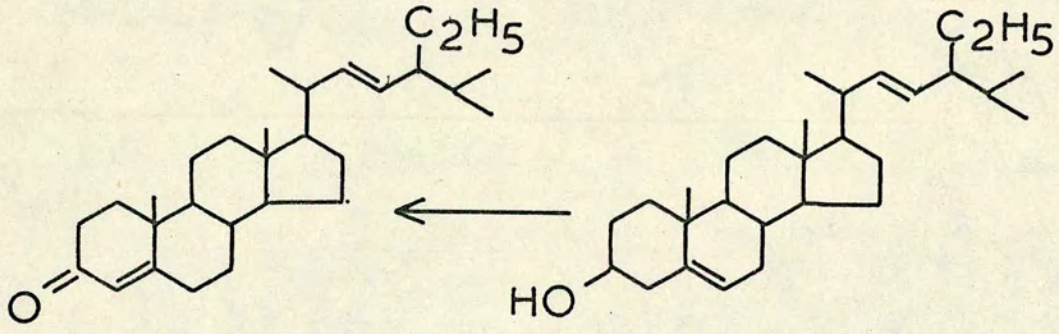
11α-Hydroxyprogesterone 11α-tosylate[86] was prepared by the



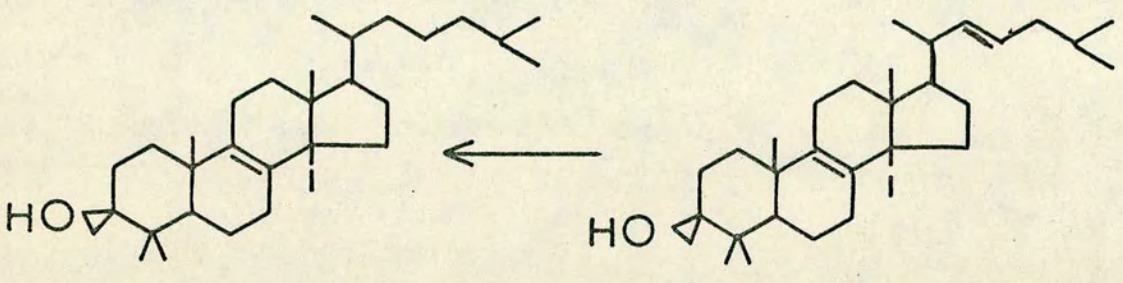
[85] [86]



[87]



[88] [89]



[90] [91]

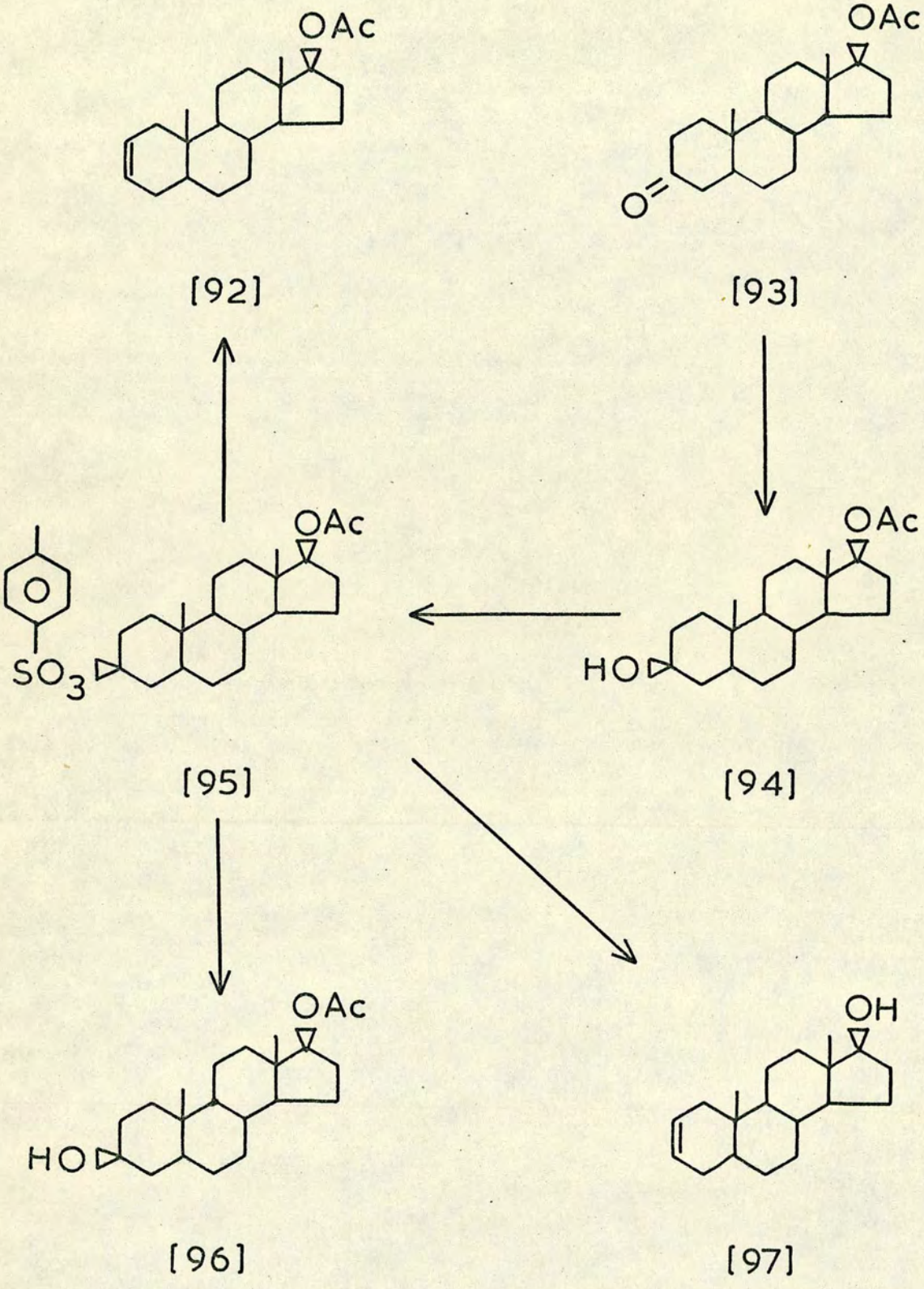
action of p-toluenesulphonyl chloride on 11α-hydroxyprogesterone [87] in pyridine in the normal esterification procedure. Less than 100% esterification occurred and it was found necessary to chromatograph the product on ethyl acetate-washed (ie neutral) alumina to obtain the pure tosylate.

Two methods were used to detosylate the product to give Δ 9(11)-progesterone. Refluxing in collidine[81] gave a high yield (66%) of the required olefin, although it was found to be difficult to remove the last traces of the collidine from the product. This, together with the high boiling point of collidine (170°), suggested it might be advisable to try another route for this stage. Refluxing of the tosylate with lithium carbonate and lithium chloride in D.M.F.[82,83] resulted in a slightly lower yield (62%) than that obtained when collidine was used, and DMF proved only slightly easier to remove during the reaction work-up. There appears to be little to choose between the two methods used.

Stigmasta-4,22-dien-3-one [88].[84,85,64]

Stigmasta-4,22-dien-3-one was prepared from stigmasterol [89] by the Oppenauer[84,85] oxidation. The keto-steroid was obtained contaminated by cyclohexanone and cyclohexanone condensation products[84,85]. The contaminants were removed by vacuum distillation using a rotary oil pump; a water pump would not provide a pressure sufficiently low to remove the last traces of contamination. The product was finally heated on a boiling water-bath in a flow of nitrogen to carry off the last of the volatile impurities.

An attempt was made to prepare the same compound using chromic acid in acetone (Jones reagent)[64]. This resulted in formation of the required compound, accompanied by several decomposition products, which were not investigated further. The N.M.R. spectrum of the product showed that the Jones oxidation gave a lower yield (40%) than the Oppenauer oxidation


OAc
[92]
OAc
O=
[93]
OAc
SO3
[95]
OAc
HO
[94]
OAc
HO
[96]
OH
[97]

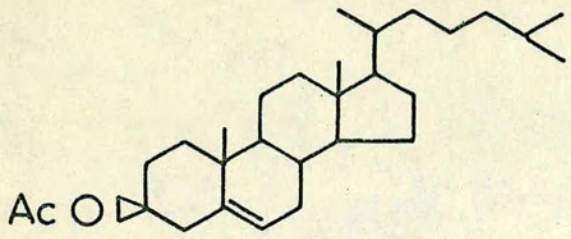
(70%) and no attempt was made to isolate the product from the Jones oxidation.

4,4,14α-Trimethylcholest-8-en-3β-ol (Dihydrolanosterol)[90][86,87,88]

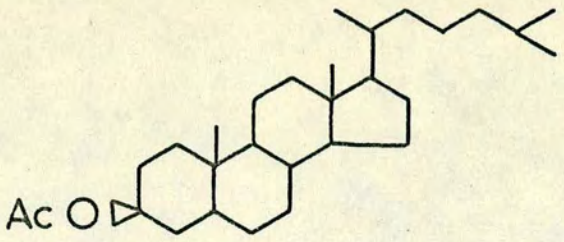
The reaction of 4,4,14α-trimethylcholesta-8,22-dien-3β-ol (lanosterol) [91] with hydrogen and a platinum catalyst resulted in hydrogenation of the C-24 double bond. The C-8 double bond is resistant to hydrogenation, as it is to the addition of hydrogen chloride[86] and perbenzoic acid peroxidation[87]. The dihydrolanosterol had M.P. 144-5° (lit[88] M.P. 145°).

Androst-2-en-17β-ol 17β-acetate [92].[89,90]

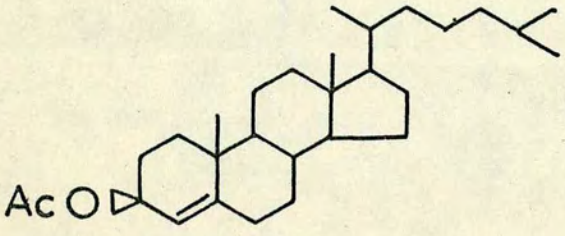
Androst-17β-ol-3-one 17β-acetate [93] was reduced in the standard manner with sodium borohydride to give androstan-3β,17β-diol 17β-acetate [94]. Tosylation of this compound gave the 3β-tosylate [95] , which by detosylation on alumina gave the Δ2-compound [92] , M.P. 97-9° (lit[89] M.P. 98-9°). Examination of the N.M.R. spectrum showed a single olefinic peak, τ 4.42, integrating for the two protons on C-2 and C-3. This would be expected from examination of the structure which shows both protons in the 2-olefin to be adjacent to a methylene group, and therefore in very similar situations. The Δ3-compound has olefinic protons in different stiuations and a more complex spectrum results. The recorded spectrum for the olefinic protons of cholest-3-ene is τ 4.35, 4.55, 4.7, 4.85[90]). The reaction gives two additional products which were identified as being the untosylated product of the reduction stage, androstan-3β,17β-diol 17β-acetate [96], and C-17 hydrolysed olefinic product, androst-2-en-17β-ol [97].



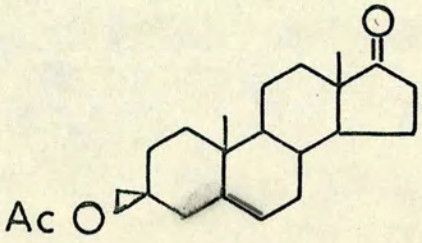
[98]



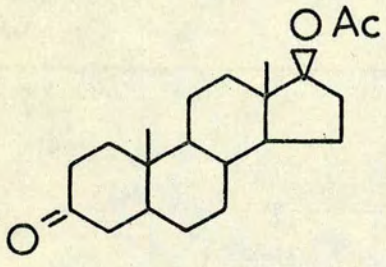
[99]



[100]



[101]



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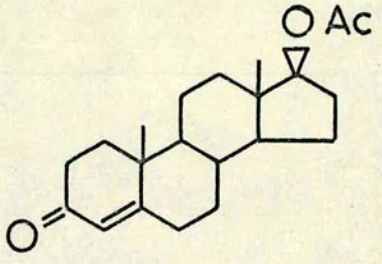

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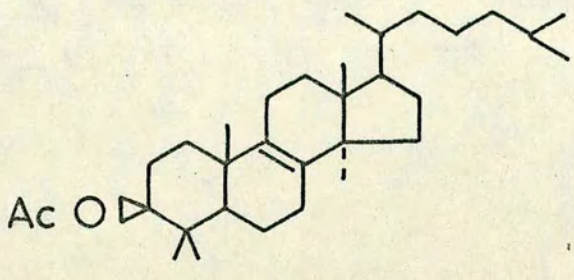


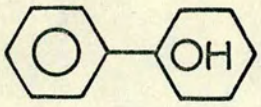
[104]

Acetates.

The following compounds were prepared by application of the standard acetylation method to the parent steroids.

Compound	Structure	Lit M.P.	Ref
Cholesteryl acetate	98	114-5°	91
Cholestanyl acetate	99	109-11°	91
Allo-cholesteryl acetate	100	83-4.5°	91
D.H.A. acetate (Dehydroepiandrosterone acetate)	101	172-3°	92
Androstanolone acetate	102	158-9°	93
Testosterone acetate	103	141-2.5°	94
Dihydrolanosteryl acetate	104	119-20°	95

In all cases, the compounds prepared had the lit.M.P.



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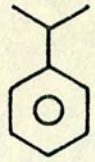
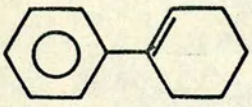
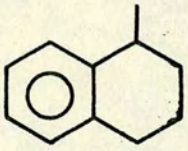


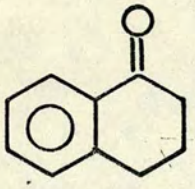
[106]



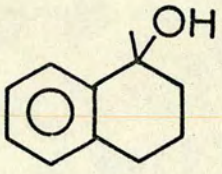
[107]



[108]



[109]



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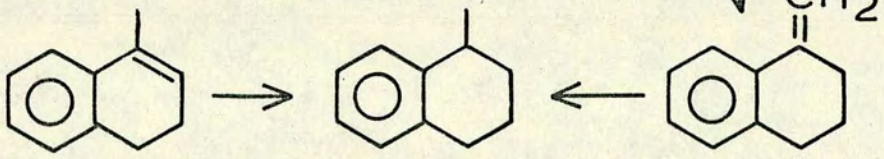
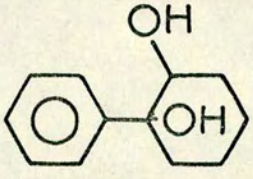


[111] [108] [112]



[113]

Non-Steroidal Aromatic Compounds.

Ethylbenzene, toluene, phenylcyclohexane, phenylcyclohexanol [105], cumene [106], and t-butylbenzene were commercial products and were reacted without purification.

Phenylcyclohex-1-ene [107]

Phenylcyclohexene was prepared by the acid catalysed dehydration of phenylcyclohexanol, using a Dean and Stark trap. Distillation at reduced pressure was used to purify the olefin which had a N.M.R. spectrum in agreement with its structure.

1-Methyltetralin [108] [96, 97, 98]

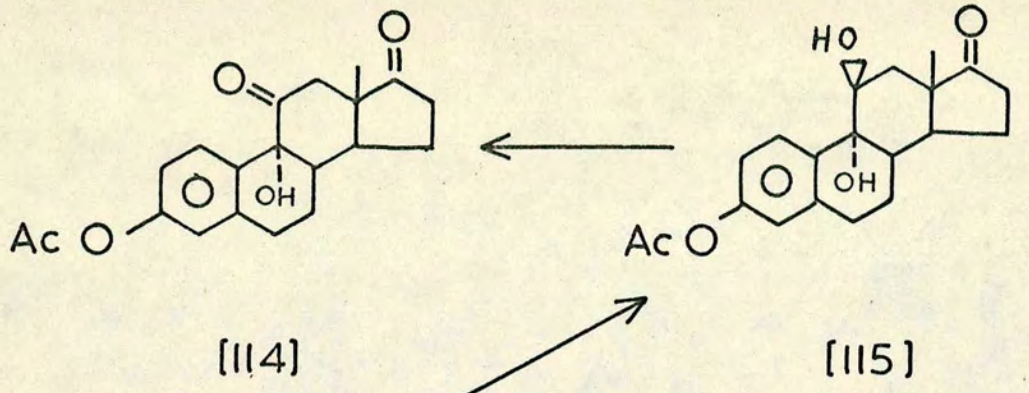
1-Methyltetralin was prepared by a three stage reaction from α-tetralone [109]. The first stage involved a Grignard reaction with methyl magnesium iodide to give the known compound, 1-methyl-1-hydroxytetralin, [110], M.P. 88-9° (lit [96] M.P. 88-9°). Acid catalysed dehydration of this compound [97] resulted in a mixture of 1-methyl-3, 4-dihydro naphthalene [111] [96,98] and 1-methylene-1,2,3,4-tetrahydronaphthalene (1-methylenetetralin) [112] [96,98] which were not separated.

The mixture was converted to 1-methyltetralin by reduction, either with sodium/amyl alcohol[98] or catalytic hydrogenation using 10% palladium on charcoal as catalyst. N.M.R. spectra confirmed that the products from both reactions were identical.

1-Phenyl-cis-cyclohexane-1,2-diol [113]. [99]

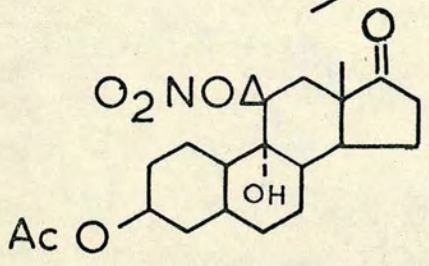
Reaction of phenylcyclohex-1-ene [107] with osmium tetroxide[99] yielded a black precipitate of the osmate ester. Hydrogen sulphide was used to decompose the ester to the required diol. After work-up, the product was obtained as crystals, M.P. 93-4.5° (lit [99] M.P. 93.5-94.5°)

It is noteworthy that only one form of this diol is known. Davies et al [99] reported that all routes, including several which normally lead to trans-diols, lead to the cis form of this compound.

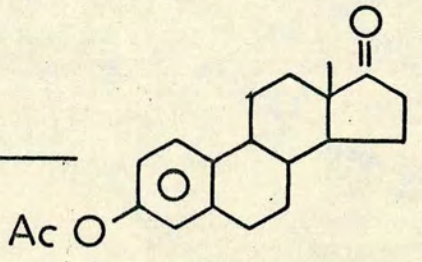


[114]

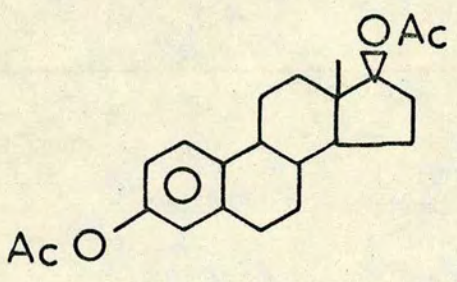
[115]



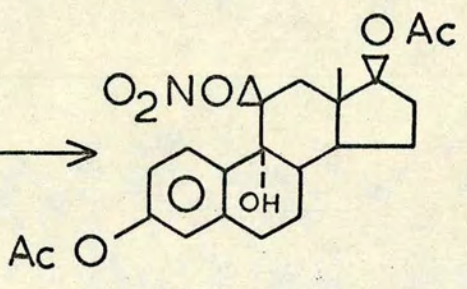
[116]



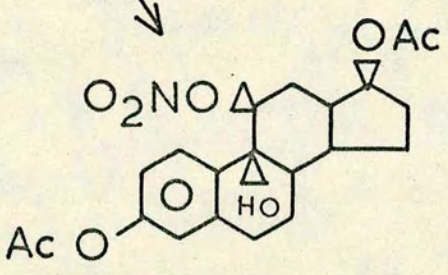
[38]



[42]



[117]



[118]

Ceric Oxidations.

CAN Oxidation of Oestrone Acetate.[100,101,64]

When CAN was reacted with oestrone acetate [38] in a molar ratio 4:1, in 90% aqueous acetic acid, a product was obtained in 70% yield, with the I.R. spectrum characteristic of a nitrate ester (νmax 1635, 1285, 865 cm^{-1}). The I.R. also showed that a hydroxyl group (νmax 3550 cm^{-1}) had been introduced into the molecule. This nitrato-steroid could be readily isolated from the reaction by crystallisation from acetone or methanol to give crystals M.P. 190-2°. Elemental analysis of this compound indicated a formula of $C_{20}H_{23}NO_7$, which is consistent with the addition of one hydroxyl group and one nitrate group to oestrone acetate. Examination of N.M.R. spectrum showed that the C-18-methyl signal had been moved downfield from τ 9.10 to τ 8.99, and a peak integrating for one proton had appeared at τ 4.21. This latter signal was assigned to the proton adjacent to the nitrate group. No signal appeared in this spectrum for a proton adjacent to a hydroxyl group (τ 5.5-6.5)[100], and this showed that the hydroxyl group was attached to a tertiary carbon atom (ie C-8, C-9 or C-14).

The nitrate compound when treated with zinc/acetic acid, zinc/aqueous ethanol, or hydrogen/10% palladium on charcoal, in each case gave a single product, whose I.R. spectrum, and elemental analysis, indicated that the nitrate ester group had been removed by hydrogenolysis. This reduced steroid, upon oxidation with chromic acid (Jones reagent)[64], gave the known compound, 3,9α-dihydroxyoestra-1,3,5(10)-triene-11, 17-dione 3-acetate [114], M.P. 247-8° (lit[125] M.P. 235-43°) indicating that the compound obtained by reduction of the nitrate ester was 3,9α, 11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate; N.M.R. of the reduction product showed a diffuse triplet (in reality a double doublet)

at τ 5.62 (CHOH, J 2.5Hz), indicating an equatorial (α) proton attached to C-11[100]. This indicated that the 11-hydroxyl group was axial (β) and the product from reduction of the nitrate ester was a 9α,11β-dihydroxy oestrone derivative [115].

The original oxidation product, the nitrate compound, also showed in its N.M.R. spectrum, a diffuse triplet (a double doublet) for the 11-proton at τ 4.21 (J ca 3Hz). This indicated again that the 11-proton was equatorial. This signal being further downfield than that for the corresponding 11-hydroxy steroid confirmed that the nitrate ester was attached axially to the 11-carbon. (This was further confirmed by the conversion of a sample of the 9α,11β-diol [115] to the nitrate ester by the action of nitric acid/acetic anhydride.)

The evidence cited above shows the CAN oxidation product of oestrone acetate to be 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate [116].

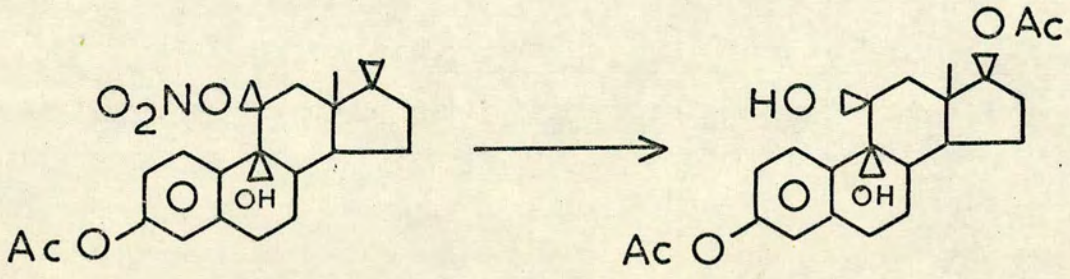
Previous reports[25,33,47,49] on the use of CAN as an oxidant in organic reactions have indicated that two moles of the reagent are required for each atom of hydrogen oxidised in the substrate. Consequently the oxidation of two hydrogens in oestrone acetate theoretically requires four moles of CAN. The highest yield of the oxidation product was however obtained when an excess of the oxidant was used. When an 8:1 molar ratio of reagent to steroid was used, 90% yield of the nitrato steroid was isolated. However it was necessary to isolate the oxidation product before all the ceric had been used, since it was found that the ceric, when present in excess, degraded the primary oxidation product and a contaminated product was consequently obtained. The highest yield (90%) of product, contaminated only with starting material ie no degradation products, was obtained when the reaction was carried out with a CAN:steroid ratio of 6:1.

The same oxidation product was obtained, but in lower yield, (40%), when 90% aqueous propionic acid was used as solvent instead of 90% aqueous acetic acid. The same product was again obtained but only in 25% yield when 90% formic acid was used as solvent.

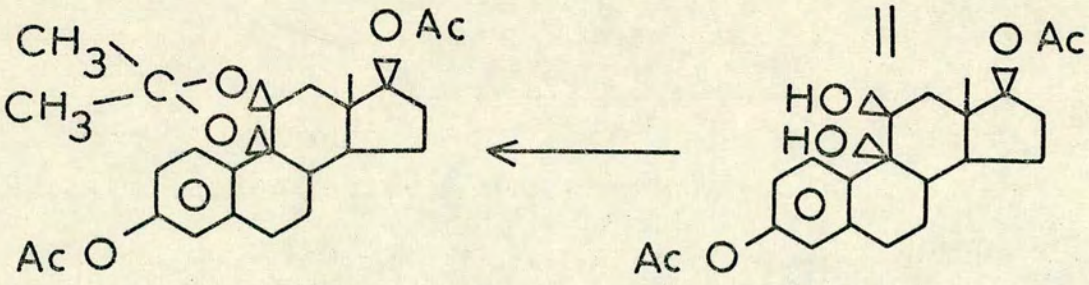
Attempts to follow the formation of the steroidal hydroxy-nitrate (using 4:1 molar ratios of CAN to steroid) by titration of the ceric with ferrous ammonium sulphate were abandoned when it was found that, as with other ceric reactions[17], the reaction was not stoichiometric. In fact the ceric was always used up before the steroid was fully reacted. This indicated that the reaction was more complex than the simple 4:1 molar ratio of ceric to steroid would suggest. Consequently titrations were only used to show when the ceric had been used up, and therefore, when no further oxidation could occur.

Structure of a Minor Product from CAN Oxidations.[76]

The CAN oxidation of oestradiol diacetate [42] gave a mixture of two products. The major product (50%) M.P. 177-8°, whose formula by analysis was $C_{22}H_{27}NO_8$, was identified as 3,9α,11β,17β-tetrahydroxy oestra-1,3,5(10)-triene 3,17β-diacetate 11β-nitrate [117] by following the same reasoning as was used for the identification of the product of the oxidation of oestrone acetate. The compound formed from oestradiol diacetate had N.M.R. spectrum, τ4.27(C-11 -proton), C-18 methyl signal lowered from τ9.18 to 9.06. The minor oxidation product (15%) with spectral properties indicating it too contained nitrate, was obtained as a syrup and upon reduction with either zinc/acetic acid or catalytic hydrogenation, gave a product which had the N.M.R. spectrum with peaks at τ5.4 (C-17α-proton), 5.6,(C-11α-proton), 6.4,(hydroxyl) and 8.86 (C-18-methyl). The C-18-methyl signal was further downfield than the corresponding signal for the 9α,11βdiol (τ8.96), obtained by reduction of the major oxidation product, and this suggested an inversion at C-9[76].



[118] [119]



[120] [119]

The compound, [119], was identified as the 9β,11β-diol by its formation of an acetonide [120](N.M.R. signals for acetonide methyls at τ8.48, 8.54) when warmed with acetone. The acetonide could not be converted back to the 9β,11β-diol. In all, three different methods to reconvert the acetonide to the diol were attempted; 90% acetic acid, 90% acetic acid containing hydrogen chloride, and aqueous ethanol containing hydrogen chloride. In each case, unchanged acetonide was returned. It is evident therefore that formation of the acetonide cannot be used to obtain the 9β,11β-diol. Formation of the acetonide of the 9β,11β-diol does however enable the isolation of pure major product, 3,9α,11β,17β-tetrahydroxyoestra-1,3,5(10)-triene-3,17β-diacetate, which cannot form an acetonide, by enabling the removal of the contaminating 9β,11β-diol, but the minor product can only be obtained by chromatography of either the initial oxidation product, the hydroxy-nitrate, followed by reduction to the diol, or by chromatography of the reduced oxidation product ie the reduced hydroxy-nitrate.

The reduction product of the minor oxidation product is therefore identified as 3,9β,11β,17β-tetrahydroxyoestra-1,3,5(10)-triene 3,17β-diacetate [119], and the minor oxidation product is 3,9β,11β,17β-tetrahydroxyoestra-1,3,5(10)-triene 3,17β-diacetate 11β-nitrate. [118] It is noteworthy that only in the case of oestradiol diacetate was the successful isolation and characterisation of this minor product carried out. Spectral evidence was obtained for the preparation of the analogous compounds for several other oestrone derivatives, namely oestrone acetate, oestrone propionate, oestrone benzoate, cholestatrienyl acetate, and oestratriene 3-acetate (17-desoxyoestrone acetate). The low yields prevented the isolation of these products. In all cases yields were lower than 10%. The compound was considered formed when the N.M.R. spectrum of the crude product contained a C-18-methyl signal,

approximately 0.20 γ lower than the parent material. The low yield prevented the location of the C-11 proton in these cases.

Products of Oestrone Derivative Oxidation.[48,102]

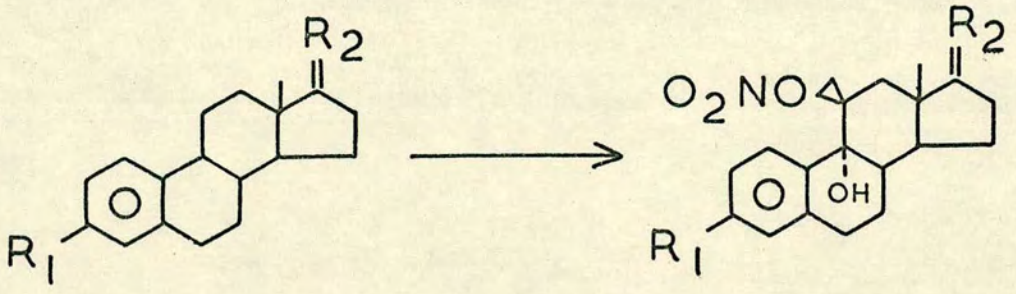
In all, sixteen variously substituted derivatives of oestrone were oxidised by CAN. In each case, examination of the N.M.R. spectrum, and analysis, showed that the corresponding 9α-hydroxy 11β-nitrate compound was formed. Yields varied between compounds and no real correlation could be found between yield and structure.

It was found that deactivating groups attached to the A-ring generally gave slower reactions, and activating groups gave faster reactions, in agreement with Murti's and Pati's[48] observations on the rate of reaction of ceric with substituted toluenes.

Use of much lower concentrations than in the preparative reactions slowed the rate of ceric uptake sufficiently to allow this to be followed by titration with ferrous ammonium sulphate, with ferroin as indicator.

3-Desoxyoestrone[53] was taken as the standard compound with which the other steroids were compared. This compound used four molar equivalents of ceric in nine hours. The activated compounds, oestrone acetate[38] , and oestrone 3-methyl ether[44] used four molar equivalents of ceric in a shorter time, 4.5, and one hour, respectively. This increase in reaction rate is in qualitative agreement with the degree of activation conferred by these groups in aromatic substitution by electrophilic reagents.

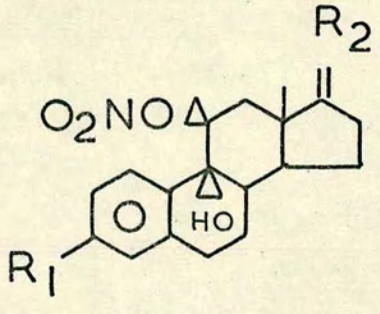
By contrast the deactivated compounds, oestrone benzoate,[43] and oestrone o-nitrobenzoate[49] used four molar equivalents of ceric in a longer time, 24, and 40 hours, respectively. Sufficient deactivation occurred in the case of 2,4-dinitrooestrone 3-methyl ether[57b] for the reaction to be stopped completely. Again these results are in agreement with the general theory of aromatic electrophilic substitution reactions.



[121]

[122]

9α, 11β



[123]

9β, 11β

The higher concentrations of steroid and CAN in the preparative oxidations again showed no reaction in the cases of 2, 4-dinitrooestrone 3-acetate [57a], and 2,4-dinitrooestrone methyl ether [57b].

The preparative scale reactions also showed the effect of activation, with oestrone methyl ether, and oestrone benzyl ether [46] reacting very quickly with ceric. The activation was found also to increase the instability of the nitrate ester, and care was needed during the work up and isolation of the ether oxidation products to prevent over-heating which resulted in extensive degradation of the product.

The oxidation of 3-hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate [39] may be considered as a special case. This compound is considered to be an intermediate (see p36) in the reaction of oestrone acetate with CAN in that the 9(11)-olefin is converted to the same 9α, 11β-hydroxy-nitrate. This olefin uses two moles of ceric in considerably less than half the time, (1.25 hours), that oestrone acetate requires to use four moles, (4.5 hours), implying that its reaction with ceric to give the final product is faster than the reaction of oestrone acetate with CAN which produces the 9(11)-olefin.

This is in agreement with previous reports[102] that oxidations of substituted toluenes have a rate-determining step of C-H bond cleavage ie cleavage of the C-9-hydrogen bond by the ceric is the rate-determining step in this reaction. (This willbe considered further in the discussion of the reaction mechanism, P 37; also 117.)

Spectral properties of the oxidation products are summarised in Table I. Compounds of general formula [121] gave products of general formula [122], except in the cases of the 2,4-dinitro derivatives.

Compounds differing from [121] in that they possessed a 9(11)-olefinic bond also gave these products.

In only two cases was an isolable product of general formula [123]

TABLE I

Substituents		Structure	SM	9α,11β-product			9β,11β product		
R_1	R_2		C_{18}	C_{18}	ΔC_{18}	11α-H	C_{18}	ΔC_{18}	11α-H
OAc	O	38	9.11	8.99	-0.12	4.21	-	-	-
OOC Et	O	41	9.11	9.00	-0.11	4.19	-	-	-
OAc	OAc_1H	42	9.18	9.06	-0.12	4.27	8.98	-0.20	3.93
OOC Ph	O	43	9.10	8.99	-0.11	4.14	-	-	-
O Me	O	44	9.12	8.99	-0.13	4.17	-	-	-
O CH_2Ph	O	46	9.11	9.00	-0.11	4.22	-	-	-
O(o-nitro-	O								
benzoyl)		49	9.10	8.99	-0.11	4.14	-	-	-
O Ts	O	50	9.12	9.01	-0.11	4.24	-	-	-
H	O	53	9.11	9.00	-0.11	4.16	-	-	-
2,4-dinitro OAc	O	57a	9.08	-	-	-	-	-	-
2,4-dinitro OMe	O	57b	9.08	-	-	-	-	-	-
OAc	H_2	58	9.27	9.16	-0.11	4.29	-	-	-
OAc		, H 64	9.29	9.16	-0.13	4.27	9.06	-0.23	3.94

obtained, and in only one of these was the product characterised.

Notes on Oestrone Acetate Oxidation.[45]

Rao[45] reported that the oxidation of benzoic acid did not proceed in the absence of light and it was found that the oxidation of oestrone acetate was also light-sensitive. When light was excluded from the CAN oxidation of oestrone acetate the reaction was found to be very much slower than when the reaction was carried out in daylight or in U.V. light (λ 354 nm).

No explanation for this effect is to be found in the literature. When the oxidation reaction was carried out at elevated temperature (80°), no increase in rate was observed, and, in fact, much degradation occurred and a lower yield of the desired product was obtained. When the reaction was carried out in solvents which had been deoxygenated by boiling, no difference was found in the overall rate of the reaction, or on the yield of product. This indicated that atmospheric oxygen played no part in the reaction.

Investigation of Mechanism of CAN Oxidation of Oestrone Acetate.

The ceric oxidation of oestrone acetate initially follows a similar route to the ceric oxidation of several other classes of compounds. When an aqueous solution of CAN was added to a solution of oestrone acetate, a dark red solution resulted, whereas CAN in either water or 90% aqueous acetic acid is orange. The dark red colour obtained, when oestrone acetate was present, showed that a complex[31,103] between the ceric species and the steroid was formed. Attempts to confirm this by measurement of the spectrum in the visible and ultra-violet regions were unsuccessful due to the very high extinction coefficient, and the very broad absorption band due to CAN (λ max.280 nm) This very large peak blotted out allother absorptions with the result that changes in the oestrone acetate spectrum, which could reveal complex formation, were unobservable. A very dilute solution of CAN was used to enable the determination of λ max, but when the calculated amount of oestrone acetate was added, the two carbonyl peaks at 276 nm and 269 nm were found to be unaltered. No change in the CAN spectrum was observed. The colour change when the reagents were mixed was however taken to be indicative of complex formation[31,103].

As mentioned earlier, titration of ceric could not be used to follow the formation of the steroid product because ceric oxidations have been found to be non-stoichiometric[17]. Titration will consequently only show the rate at which ceric disappears. The time to total disappearance can however be used to give a qualitative measure of the overallrelative reaction rates of oestrone derivatives. In agreement with the results of Murti and Pati[48], it was found that the presence of activating groups in ring A results in faster reaction ie faster consumption of ceric. By contrast, the presence of deactivating groups results in slower reaction, again as measured by total uptake of ceric.

These observations are in accord with the general theory for the rates of reaction of electrophiles with aromatic systems. The observed rates of these CAN oxidations suggests that the attacking ceric species is of cationic character. Unfortunately the precise nature of the ceric species in the reaction is not known, although it has been reported[104] that ceric nitrate species are dimeric even in dilute solution. It is also known that ceric in solution is 8-co-ordinated[28], but other than this the precise nature of the oxidative species is not known.

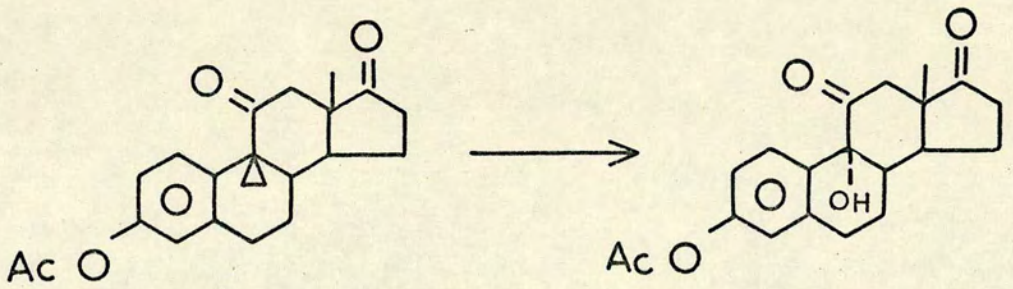
The ceric species, after complexing with the oestrone acetate operated in itsusual manner[102] by abstracting a benzylic hydrogen to yield a hydrogen ion. As expected, abstraction of the 9-tertiary hydrogen to yield a tertiary radical is favoured over removal of a secondary benzylic hydrogen from C-6, to yield a secondary radical. No product which could be associated with a C-6 benzylic radical has been observed in these reactions. Attempts were made to prepare oestrone acetate substituted with a 9α-fluorine [76]. It was felt this would prevent reaction at C-9 and possibly result in reaction at C-6. All preparations were unsuccessful.

Oxidative attack by ceric at the benzylic position is a common feature of the reaction of all aromatic compounds which possess a benzylic hydrogen or conjugated olefin eg Δ9(11)-oestrone acetate. Oxidation of such compounds readily leads to one or two major products only[47,50]. In particular, 9β-oestrone acetate [40] gave the same oxidation product as oestrone acetate, confirming the formation of a common benzylic radical derived by removal of either a 9α- or 9β-hydrogen. By contrast, aromatic compounds which do not possess a benzylic hydrogen or olefin were not oxidised. For example, t-butyl benzene is unaffected by CAN, and non-aromatic compounds undergo extensive degradation giving up to fifteen unidentifiable products,

formed by oxidation of the carbon skeleton by ceric.

The radical nature of the initial step was confirmed by the reaction between CAN and oestrone acetate being stopped by the addition of acrylamide. Acrylamide is known to be a radical scavenger[105]. Acrolein was a less efficient scavenger and only slowed the reaction down, and reduced the yield of oxidation product.

The 9-radical once formed is envisaged as then reacting along any of the following pathways. The benzylic radical may react with water to abstract an H-atom, thereby regenerating starting material. This reaction may well in turn account for the observed non-stoichiometry of this reaction. The radical may however react with a species such as Ce(IV)OH L_7[40] leading to a 9-hydroxy steroid. Although 9-hydroxyoestrone acetate [71] has never been isolated as an intermediate for this reaction its observed instability to strong acid, which causes its rapid dehydration to the 9(11)-olefin[106], could account for not observing the compound directly. 9α-hydroxyoestrone acetate is however oxidised by CAN to the same 9α-hydroxy-11β-nitrate derivative of oestrone acetate as is oestrone acetate itself. The presence of a species such as Ce(IV)OH L_7 in this reaction is however by no means certain, and the 9-benzylic radical may be further oxidised to a 9-carbonium ion by another 1-electron transfer reaction by Ce(IV)L_8[28]. Two consecutive 1-electron transfer reactions by Ce(IV) to yield a cation has been reported by Dewar et al[43], and Trahanovsky et al[44] to explain the formation of tropylium salts during the oxidation by CAN of cyclohepta-2,4,5-triencarboxylic acid [19] and cyclohepta-1,3,5-triene respectively [22]. The 9-carbonium ion may either react with a water molecule to give the same 9α-hydroxy steroid as postulated above, or more likely, lose an 11β-hydrogen in an elimination reaction to yield the 9(11)-olefin. The latter would in any case arise by the facile dehydration of the



[124] [125]

9α-hydroxy compound in the strong acid conditions of the reaction. It is of interest to note that the fully substituted 8(9)-olefin was never observed as a reaction intermediate. However it has been reported[107] that the 8(9)-olefin readily isomerises under acidic conditions to give the Δ9(11)-steroid.

A careful examination of the reaction products of the ceric oxidations of the two proposed intermediates suggested that the 9(11)-olefin is the more likely of the two, since the 9(11)-olefin is oxidised at a rate which is both faster than the rate of oxidation of the 9α-hydroxy steroid and the rate of dehydration of the hydroxy compound to the olefin. As previously mentioned no 9α-hydroxy compound was ever isolated from these reactions, unless present initially. By contrast, the 9(11)-olefin could be isolated from the oxidation of the 9α-hydroxy compound.

The two compounds discussed above were proposed as intermediates only because they gave the same final oxidation product as oestrone acetate when treated with CAN, although neither has actually been isolated during the ceric oxidation of oestrone acetate itself. This suggests that olefin formation and its subsequent oxidation could occur as a concerted reaction but no method could be found to confirm this.

The further reaction of the 9-benzylic radical with ceric to yield a 9α-hydroxy steroid was however observed directly during the oxidation of 11-keto-isooestrone acetate [124]. In this ceric oxidation, the principle oxidation product was 3,9α-dihydroxyoestra-1,3,5(10)-triene-11,17-dione 3-acetate [125], which arises due to the impossibility of eliminating an 11-hydrogen, although why a hydrogen does not eliminate from C-8 is not clear. These observations confirm that the formation and subsequent reaction of the 9-radical is an integral part of the oxidative reaction sequence.

Reaction of the 9(11)-olefin, 3-hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate or the 9α-hydroxy steroid, 3,9α-dihydroxyoestra-1,3,5(10)trien-17-one 3-acetate, with CAN in the presence of acrylamide resulted in formation of the normal product, 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate showing that this stage of the reaction does not involve a radical reaction. This ceric oxidation is performed by a ceric species of unknown composition. As mentioned earlier, ceric can exist in dimeric, and polymeric[104] forms and ceric in solution is 8-coordinated[28]. The composition of the eight ligands is however not known for the system used in these CAN oxidations. Two possible reaction pathways can be envisaged to account for the oxidation of the 9(11)-olefin to the final product. An unknown ceric species could complex to the less sterically hindered α-face of the Δ9(11)-steroid. This would allow a nitrate-containing species to attack the steroid at the β-face to yield exclusively the observed 11β-nitrate. From none of the reactions studied has an 11α-nitrate ester ever been observed. An intermediate species, occurring during nitrate entry, by undergoing a complex oxidative step involving two 1-electron transfers, would eventually lead to the formation of a 9-carbonium ion. Attack at the 9-position by water then yields the observed 9α-hydroxy-11β-nitrate as the final oxidation product. In all cases however a small amount of the 9β-hydroxy-11β-nitrate was also observed. The non-stereospecificity occurring during this step would support the formation of a planar carbonium ion at C-9.

The observed stereospecificity, of the 11β-nitrate group, which occurs in these reactions, despite the proximity to the axial C-18 methyl group, suggests an alternative reaction pathway. The nitrate group could be considered as entering the steroid by ligand transfer from ceric. A ceric species, such as the known $H_2Ce(NO_3)_6$[30], could complex to the

β-face of the steroid, 9(11)-dehydrooestrone acetate, and ligand transfer occur to give only 11β-nitrate addition. Subsequent oxidation by ceric could result in the formation of the C-9 carbonium ion which by normal reaction with water would give the observed products. As stated before, the composition of the oxidising specieswith respect to the numbers of ceric ions, and ligands, is not known. It would be expected that acetate groups could complex to the ceric and could consequently be transferred to C-11 if ligand transfer is indeed involved in the introduction of the group entering at C-11. In fact no acetate has ever been observed to have been incorporated. This is believed to be due to the low pH of the solution, (pH≏0) caused by the presence of free nitric acid, which results in most, if not all, of the acetic acid, in the reaction medium, remaining unionised. Consequently few, or none, of the ceric ligands are acetate, and therefore incorporation into the steroid molecule cannot occur.

The oxidation of toluene which gives a high yield of benzyl acetate[49] would appear to disagree with the above reasoning. This reaction however is not comparable with the oxidation of oestrone derivatives in that the benzylic carbon is considerably less sterically hindered than that of the steroid molecule. The carbonium ion once formed can react readily with a solvent species, acetic acid, nitric acid, nitrate or water to yield the products, benzyl acetate or benzyl nitrate. Reaction with water yields benzyl alcohol which can further react to yield benzaldehyde, another product. By comparison, the steroid ion is too sterically hindered to react with any species other than water. Reaction with acetic acid would result in a tertiary acetate, which are known to be difficult to prepare. The steroid carbonium ion either reacts with water to give the 9α-hydroxy compound which dehydrates to the Δ9(11)-compound, or yields this compound directly by loss of hydrogen from C-11.

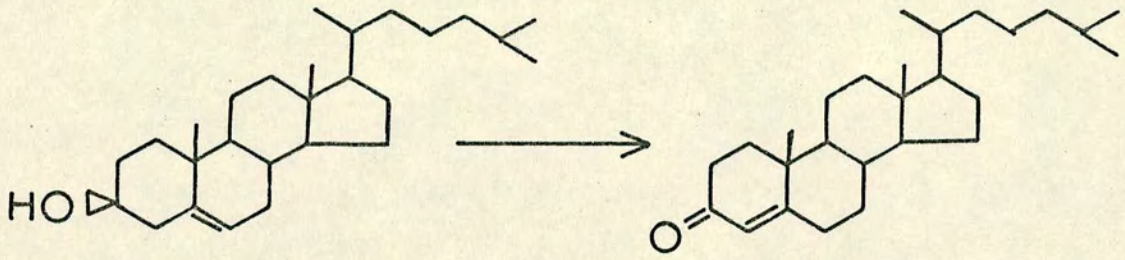
Subsequent study of the 9α-hydroxy 11β-nitrate derivative of oestrone acetate showed that treatment with weakbase, potassium acetate or potassium bicarbonate, resulted in the formation of 3-hydroxy-9α,11-epoxyoestra-1,3,5(10)-trien-17-one 3-acetate [72]. This suggested that the epoxy-steroid could be a reaction intermediate, which by further oxidation by ceric or by addition of nitric acid yielded the isolated product.

Treatment of the 9α,11-epoxy compound with CAN, or with nitric acid resulted in both cases only in formation of 3-hydroxy-9β-oestra-1,3,5(10)-triene-11,17-dione 3-acetate [124], which showed that this epoxide was not an intermediate between the 9(11)-olefin and the final oxidation product, the 9α-hydroxy-11β-nitrate.

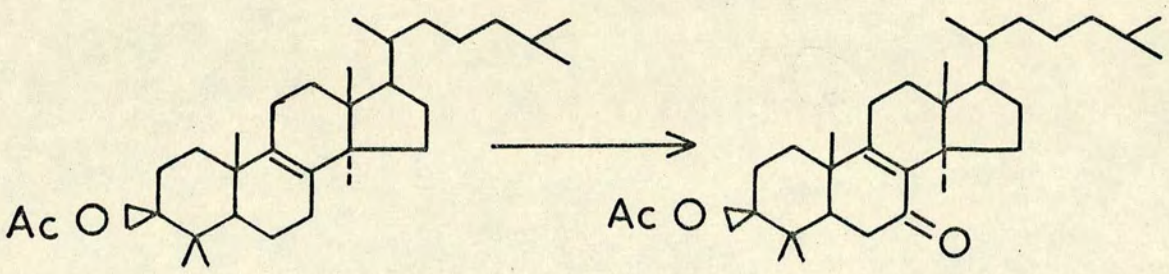
A possibility for an intermediate stage between the 9(11)-olefin and the final oxidation product could be an epoxide from the reaction of the 9(11)-olefin with peracetic acid. Peracetic acid might have possibly arisen from OH radicals produced by oxidation of water by CAN. No peracetic acid hasactuallybeen found in the reaction medium, but if present in low concentration, it could possibly effect this reaction ie formation of an epoxide. The 9(11)-olefin, when stirred for fifteen minutes in 90% acetic acid with a ten-fold excess of peracetic acid, prepared by the addition of hydrogen peroxide to acetic acid[108], produced no epoxide. A previously reported[75] method of preparation of 9β,11-epoxyoestrone acetate [73] was found to yield only the 9α,11-epoxide and, therefore, the possibility that the 9β,11-epoxy compound was an intermediate could not be investigated. These experiments showed the CAN oxidation of oestrone acetate did not involve an epoxide intermediate.

The evidence listed above suggests the following reaction sequence for the CAN oxidation of oestrone acetate; An unknown ceric species complexes to the steroid, with the initial oxidation occurring at C-9

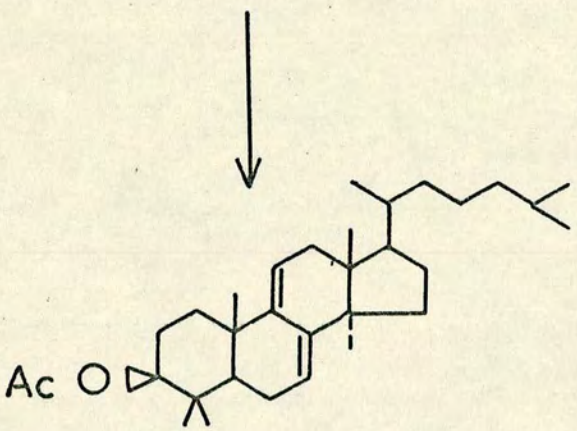
to yield a benzylic radical which by subsequent oxidation and reaction yields the 9(11)-olefinic compound, 9(11)-dehydrooestrone acetate. This compound then further reacts with a ceric species (again of unknown form) to incorporate an 11β-nitrate group, and to become oxidised to a planar C-9 carbonium ion, which by reaction with water gives the 9α-hydroxy-11β-nitrate compound, although this attack at C-9 is not completely stereospecific in that the 9β-hydroxy-11β-nitrate compound is also observed. This mechanism agrees with all the evidence assembled to date on this reaction.



[126] [81]



[104] [127]



[128]

Essential Requirements for the Ceric Oxidation Reaction.

The ceric oxidation reaction was studied to determine which of the following were essential for reaction to proceed:

1. Aromatic ring.
2. Benzylic hydrogen.
3. Ceric.
4. Nitrate ions.
5. Ammonium ions.
6. Water.
7. Acidic solvent.

Requirement of an Aromatic Ring.[95]

CAN oxidations of non-aromatic steroids in all cases resulted in either extensive degradation or the production of previously known normal oxidation products. For example, cholesterol [126] gave cholest-4-en-3-one[81], the yield being very dependent upon the molar ratio of ceric used. When excess ceric was used, extensive degradation, and a lower yield of product, was observed. Dihydrolanosteryl acetate [104] gave a mixture of 7-ketodihydrolanosteryl acetate [127], and dihydrolanosta-7,9(11)-dienyl acetate [128], both of which are normal[95] oxidation products of this compound. By contrast, D.H.A. acetate[101], and testosterone acetate [103] gave only unreacted starting materials, and unidentifiable degradation products. In contrast to these observations, whenever a steroid containing an aromatic ring was oxidised with CAN, the products were few in number and easily identified. A series of oestrone derivatives illustrated this best, where sixteen variously substituted steroids gave, in no case more than two primary products, though in the case of the very fast reacting oestrone ethers partial degradation of the primary products occurred.

Non-steroidal compounds containing an aromatic ring also reacted

smoothly with CAN to give a small number of identifiable products. The oxidation of phenylcyclohexane and its simple derivatives (see P 56.) suggested that the fixed stereochemistry of the aromatic steroid skeleton helped to minimise the number of products formed, since in all cases, these compounds gave more minor products than the rigid steroids. These non-steroidal compounds gave oxidation products comparable in structure to the products resulting from the oxidation of steroids ie diols, nitrate ester etc.

Comparison of two steroids similar in structure but with one possessing an aromatic ring and the other without this feature also showed the necessity of the aromatic ring for the reaction to yield a small number of simple oxidation products. Reaction of androst-2-enyl acetate [92] with CAN resulted only in extensive degradation of the olefinic bond. The N.M.R. spectrum suggested that much hydroxyl incorporation had occurred during oxidation, but the only isolable product was unreacted starting material. By contrast, 3-phenylandrost-2-enyl acetate [65] gave the normal ceric oxidation product, readily isolable and identifiable by infra red and N.M.R. spectra as 3β-phenyl-2β,3α,17β-trihydroxyandrostane 17β-acetate 2β-nitrate. [130]

Requirement of Benzylic Hydrogen.

All compounds which reacted relatively cleanly with CAN possessed a benzylic hydrogen or benzylic olefinic group. That this was essential for the reaction was confirmed by the non-reaction of t-butyl benzene. A small amount of degradation occurred but even with excess CAN, almost all of the compound was returned unreacted.

Attempts to prepare 9α-fluorooestrone acetate [76] by several different routes were unsuccessful. Reactions of this compound could have confirmed the necessity of tertiary benzylic hydrogen in the steroid series for the preparation of the 11β-nitrate compound. It would have

been of interest to note whether CAN oxidation of the 9α-fluoro steroid would have occurred at C-6, this being the other position containing benzylic hydrogens, thus by-passing the requirement of reaction at C-9.

Requirement of Ceric.

The reaction between oestrone acetate and nitric acid gave, as product, unreacted starting material, contaminated with a very small amount of hydrolysed acetate. Repetition of the reaction in the presence of cerous ions (cerous acetate) again resulted in no oxidation of the oestrone acetate, as did the reaction between oestrone acetate and nitric acid with the addition of perchloric acid. These three reactions showed that the first stage of the reaction requires ceric ions for oxidation to occur.

The assumption that 3-hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate [39] or 3,9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate [71] are possible intermediates enabled similar study to be made of the second stage of the oxidation reaction. The 9(11)-olefinic compound when reacted with nitric acid gave as the only product unreacted starting material, indicating that in the absence of ceric no reaction had occurred. The 9α-hydroxy compound when treated exactly as the 9(11)-olefin gave a mixture of two products, unreacted starting material and the expected dehydration product, 3-hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate.

Requirement of Nitrate Ions.

The reaction between oestrone acetate and several ceric compounds was studied. These ceric salts were ceric hydroxide, ceric ammonium sulphate, and ceric sulphate. In each case, the only product isolated after the oxidation reaction was starting material, though streaking on the T.L.C. plates showed that some degradation had occurred. No oxidation product could be isolated or identified from any of the

reactions. In each case, difficulty was experienced in keeping the ceric in solution. Ceric sulphate is soluble in dilute sulphuric acid, but, on dilution with acetic acid to ensure solution of the steroid, the ceric immediately precipitated to give a pale yellow suspension. No solvent from 50% acetic acid up to 100%, could be found which retained the ceric in solution. Filtration of the suspension gave a yellow solution showing that some ceric remained in solution. These reactions were therefore carried out using the suspensions of ceric obtained.

Repetition of these reactions with solution of the ceric compounds in nitric acid prior to the addition of the steroid solution resulted in two changes; the ceric remained in solution, after addition of the steroid, to give a yellow, or orange, solution, and the steroid was oxidised to give the normal ceric oxidation product, 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate, 11β-nitrate, in moderate yield (50-60%). This showed that the presence of nitric acid is essential for ceric oxidation of oestrone acetate to occur.

The reaction was repeated with ceric hydroxide, as oxidative species, dissolved in concentrated hydrochloric acid. The ceric dissolved to give a red solution and after addition of the steroid solution, the ceric remained in solution. No oxidation product of the steroid was isolable. When the reaction was repeated, but with the addition of ammonium nitrate, a small amount of the 9α-hydroxy-11β-nitrate compound was obtained. The same oxidation reaction with ceric hydroxide in perchloric acid also gave unchanged starting material, with degradation, but, in perchloric acid which contained ammonium nitrate, a small amount of the nitrate ester was obtained. These reactions indicated that nitrate ions were essential for the clean oxidation by ceric of oestrone acetate. The fact that the nitrate ions could be added as the ammonium

salt, showed that the nitrate was present not only to facilitate solution of the ceric, but also to form the complex oxidising species, by combination with ceric, required for the successful oxidation reaction.

Requirement of Ammonium Ions.

The reactions of ceric hydroxide, and ceric sulphate, with oestrone acetate, in the presence of nitric acid, which give the 9α-hydroxy-11β-nitrate compound, showed that the presence of ammonium ions is not necessary for the oxidation to occur.

Requirement of Water.

Anhydrous acetic acid, and anhydrous methyl ethyl ketone were found to be moderately good solvents for CAN. Both solvents dissolved the reagent, although stirring for several hours was necessary to ensure total solution. When these solutions of oxidant were reacted with oestrone acetate, the only isolable product consisted of unchanged starting material, though T.L.C. of the crude product showed a very faint shadow corresponding to the 9α-hydroxy-11β-nitrate compound. This undoubtedly arose from the small quantity of water which would be present in the dried solvents, or in the CAN itself.

Requirement of Acidic Solvent.

The reaction was carried out in several solvents, which were water miscible to a greater or lesser degree.

Benzene and ether are both immiscible with water, and when used as solvents, only unreacted starting material was obtained after the reaction. Methyl ethyl ketone and water were only partially miscible, but the reaction was vigourously stirred to encourage mixing, and a moderate yield (40%) of the 9α-hydroxy-11β-nitrate compound was obtained. Acetone gave a similar yield (40%) of this same product.

Dioxan as solvent gave a lower yield (25%) but the product was

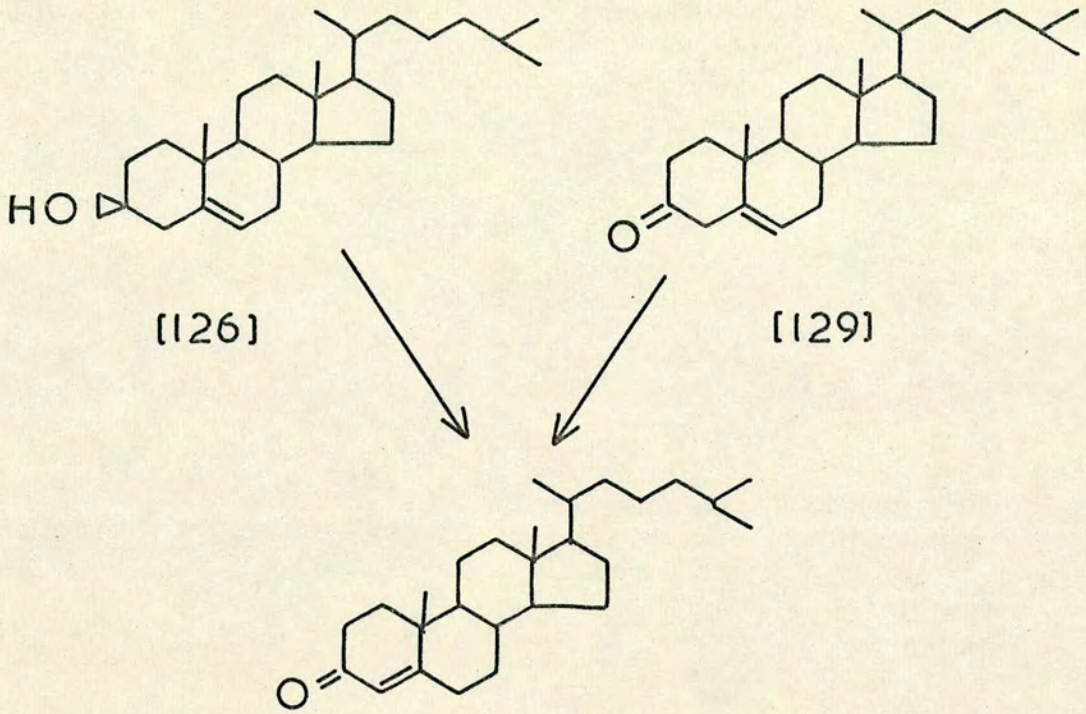
still isolable as crystals. THF, however, although also an ether with similar solvent properties, gave the product in a quantity (5%) which could not be crystallised. The product was identified by T.L.C., and I.R., and N.M.R. spectra. Several other solventsgave the product in quantities which were only detectable by T.L.C., and I.R., and N.M.R. spectra, but not isolable as crystals. These were t-butanol, dimethylformamide, and dimethylacetamide. Methanol was found to give none of the expected product.

The difference between methanol, giving no product, and t-butanol, giving a small yield (5%), can be explained by the ceric oxidising the methanol in preference to the steroid. t-Butanol by contrast requires more drastic conditions to oxidise it since a carbon-carbon bond rupture is required for oxidation to occur.

Although not all of the solvents gave positive results, a sufficient number did so to show that an acid solvent is not necessary for the CAN oxidation of oestrone acetate to occur. An acidic solvent does however result in higher yields.

Conclusion.

It is apparent therefore that the ceric oxidation reaction requires the compound to be oxidised to possess an aromatic ring with a benzylic hydrogen, or an olefinic bond adjacent to the ring, and the reaction medium to contain ceric ions, nitrate ions and water. Ammonium ions and an acidic solvent are not required though an acidic solvent does result in higher reaction yields.



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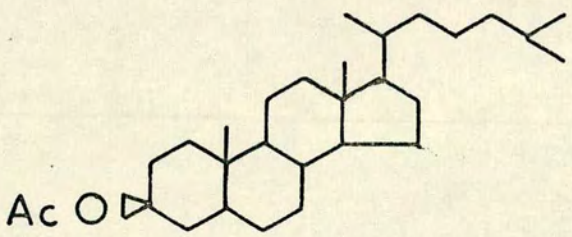

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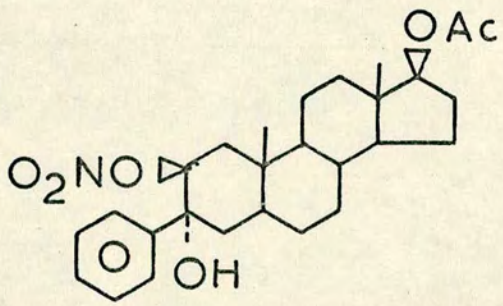


[130]

Oxidation of Non-Aromatic Steroids[95]

Several non-aromatic olefinic steroidswere reacted with CAN (See Table II) in the hope that allylic oxidation might occur to give a hydroxy group adjacent to the double bond. These steroids were found to be either unaffected by the reagent or extensively degraded. The latter indicated allylic oxidation could be occurring but further reaction took place too fast for any product to be isolated. Oxidation of the double bond itself to give diols, diones etc could also account for degradation of the substrate.

However, during the oxidation of dihydrolanosteryl acetate [104], two products could be readily isolated from the reaction. These were the two known compounds, expected from the oxidation of the 8(9)-tetra-substituted double bond[95], namely dihydrolanosta-7,9(11)-dienyl acetate [128], and 7-ketodihydrolanosteryl acetate [127].

Both cholesterol [126] and cholest-5-enone [129] were converted to cholest-4-enone [81] by reaction with CAN. In the case of cholesterol, a large excess of Ce(IV),molar ratio 20:1, showed that once degradation of the molecule began, Ce(IV) was able, relatively easily, to oxidise the steroid chain progressively giving a multiplicity of products, and, in the long term, would probably convert the steroid completely to carbon dioxide. After a reaction involving CAN with cholesterol in a 20:1 molar ratio, cholest-4-enone could stillbe isolated, (40%), although all the ceric was eventually used, presumably to produce extensive degradation of the cholesterol.

The case of the saturated steroids, androstanolone acetate [102], and cholestanyl acetate[99], which were both oxidisedby ceric, showed that the steroid skeleton itself was susceptible to ceric oxidation. These compounds, although reacting slower than their olefinic counterparts, were still oxidised by ceric. The probable sites of attack are, adjacent

to the carbonyl position in the case of androstanolone acetate, and adjacent to the acetate group in both compounds. No identifiable oxidation products could be isolated from these reactions.

Different ratios of CAN: steroid for different periods of time were reacted in an attempt to obtain isolable products. The results of these experiments are summarised in Table II.

TABLE II

Compound	Ceric (mole equivalents)	Structure	Duration of Reaction (hours)	Products (% yield or return)
Cholesterol	2	126	18	Cholest-4-enone (80%)
"	4		18	" (70%)
"	20		96	" (40%)
Allocholesterol	2	80	18	Cholest-4-enone (75%)
"	4		18	" (70%)
Cholest-5-enone	4	129	18	Cholest-4-enone (52.5%)
Cholest-4-enone	2	81	18	Cholest-4-enone (50%)
"	2		3	" (90%)
Cholesteryl acetate	2	98	18	Cholesteryl acetate (50%)
"	4		18	" (40%)
Allocholesteryl acetate	2	100	4	Allocholesteryl acetate (50%)
Cholestanyl acetate	2	99	60	Cholestanyl acetate (70%)
Dehydroisoandrosterone	2	101	6	D.H.A. acetate(60%
(D.H.A.) acetate	2		15	" (50%)
"	2		1½	" (80%)

Compound	Ceric (mole equivalents)	Structure	Duration of Reaction (hours)	Products (% yield or return)
Testosterone acetate	2	103	18	Testosterone acetate (70%)
Δ9(11)-Progesterone	2	85	18	9(11)-Progesterone(70%)
	2		15 mins	" (90%)
	8		15 mins	" (90%)
	8		1	" (90%)
Androst-2-enyl acetate	2	92	18	Androst-2-enyl acetate (80%)
Stigmastadienone	2	88	18	Stigmastadienone (80%)
Dihydrolanosteryl acetate	4	104	15 secs.	Dihydroagnosteryl acetate (30%) 7-keto dihydro-lanosteryl acetate (20%)
Androstanolone acetate	4	102	18	Androstanolone acetate (15 %)
"	2		4	" (15%)
"	4		8	" (25%)

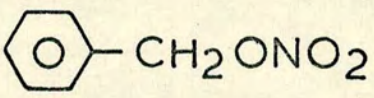
Comparison of Androst-2-enyl Acetate and 3-Phenylandrost-2-enyl Acetate Oxidations.

In common with all but three, (dihydrolanosteryl acetate, cholesterol and cholest-5-enone), of the non-aromatic steroids, androst-2-enyl acetate, when reacted with CAN, yielded starting material as the only isolable product.

By contrast, 3-phenylandrost-2-enyl acetate reacted with CAN to give a hydroxy-nitrate derivative[130] (M.P. 190-5°, yield 60%), indicated by the appearance of nitrate peaks at ca 1640, 1280, 860 cm^{-1} in the I.R. spectrum. The presence of a nitrate ester group was confirmed by the downfield shift of the C-19 methyl peak of the androstane, from τ 9.22 to 9.11. Comparison of the structure here shows that the geometry existing between the C-19 methyl and the 2 -nitrate in the compound is similar to that of the C-18 methyl and the 11β-nitrate groups in the oestrone acetate oxidation product, and therefore a similar shift in N.M.R. signal of about 0.11τ would be expected. Examination of the N.M.R. spectrum also shows a peak at τ 9.19; this was attributed to the C-18-methyl signal which by incorporation of the 3α-hydroxyl, and 2β-nitrate ester groups has undergone a downfield shift of τ 0.06, from τ 9.25 to 9.19. The starting material for this oxidation consisted of an 85:15 mixture of the 3-phenylandrost-2-enyl acetate, and 3-phenylandrost-3-enyl acetate. This it was felt could result in a mixture of products, but in view of the M.P. and yield of the derived hydroxy-nitrate it was assumed that this oxidation product arose solely from the 2-olefin. Any product arising from oxidation of the Δ3-steroid was separated by crystallisation and was not isolated.

The mechanism of this oxidation reaction appears to parallel exactly the mechanism proposed for the oxidation of oestrone acetate. It is

interesting to note that the free rotation of the 3-phenyl group does not evidently alter the course of the reaction.



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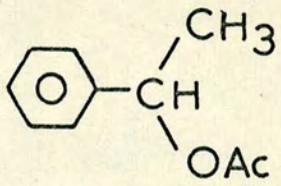

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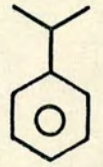

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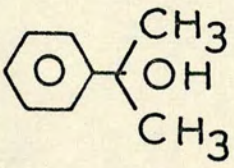

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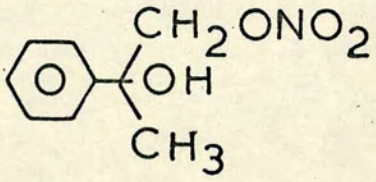

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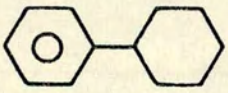

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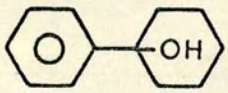

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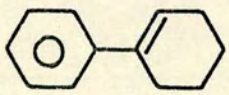

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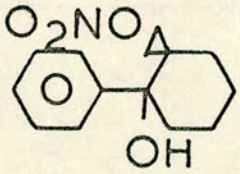

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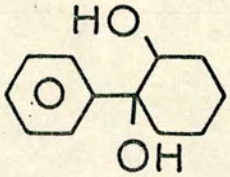


[137]

Non-Steroidal Aromatic Compounds.

A series of aromatic compounds was oxidised with CAN using acetic acid as solvent. This was partly todetermine whether CAN oxidation to yield nitrate esters occurred only when the oxidised molecule possessed the rigid steroid stereochemistry or whether flexible molecules could be similarly oxidised, and partly because the starting materials were easier to prepare than some of their steroid counterparts. In particular, this latter applied to the oxidation of t-butyl benzene which was carried out to determine whether a tetra-substituted benzylic carbon atom could be oxidised. Attempts to prepare a tetra-substituted oestrone derivative (9α-fluorooestrone acetate [76]) had been unsuccessful.

The series of compounds consisted of toluene, ethylbenzene, cumene, t-butylbenzene, phenylcyclohexane, phenylcyclohex -1-ene [107], phenylcyclohexan-1-ol [105], and methyl tetralin [108].

Toluene[50,47]

When toluene was oxidised in the usual manner with CAN, using 90% acetic acid as solvent, a high yield of benzyl nitrate [131] was obtained. This was confirmed by the presence of nitrate peaks in the I.R. spectrum, which could be readily removed by zinc/acid reductionto yield benzyl alcohol; a reaction again confirmed by N.M.R. spectroscopy. A minor product was benzyl acetate, confirmed by comparison of the N.M.R. spectrum with that of the authentic compound. This result is in agreement with that of Dust and Gill[50] who also isolated benzyl nitrate from this reaction, but reported that the ester was rapidly hydrolysed to allow further oxidation to benzaldehyde to occur. The two products obtained here indicated that the reaction follows the usual path; one electron oxidation to yield a benzyl radical which, after further oxidation to give a carbonium ion, reacts with a solvent species, nitric or acetic acid, to yield the product.

When the reaction was repeated using the same conditions as Syper [47] used, results in agreement with his were obtained ie a high yield of benzaldehyde and no benzyl nitrate.

Ethylbenzene.

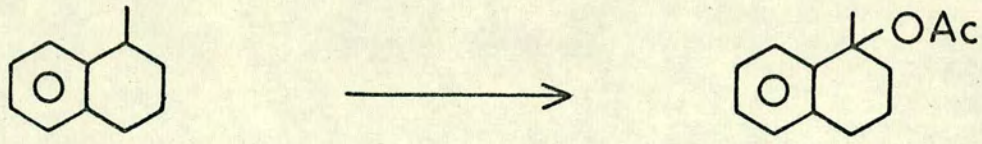
CAN oxidation of ethylbenzene with 90% acetic acid as solvent resulted in two major products. These were acetophenone, and α-phenyl ethyl acetate [132], with a small quantity of α-phenyl ethanol, which was designated as being the precursor to acetophenone. No nitrate ester was formed. This result again confirmed the mechanism as being one electron oxidation to yield a radical, here a secondary radical, which after a second oxidation to give a carbonium ion reacted further with a solvent species, here water or acetic acid to give the product, the alcohol or acetate [132]. No nitrate incorporation occurred, presumably due to steric crowding preventing the large nitrate group entering so close to the aromatic ring.

Cumene.

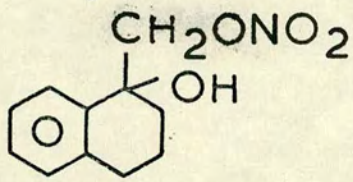
Oxidation with CAN in 90% acetic acid yielded a mixture of products comprising cumyl alcohol [133] and 2-phenyl propane-1,2-diol 1-nitrate [134]. This showed the reaction again formed a radical intermediate which could however react in two ways; further oxidation and reaction with water to yield cumyl alcohol, or a reaction analogous to the steroid reactions to yield a hydroxy nitrate. This showed that the rigid steroid stereochemistry was not essential for formation of a hydroxy-nitrate product but the rigid stereochemistry did result in higher yields of the nitrate product.

t-Butyl benzene.

As expected, t-butyl benzene did not react with CAN. A small amount of degradation did occur but the high yield of returned starting material showed that a benzylic hydrogen was essential for a successful oxidation reaction to occur.



[108] [138]



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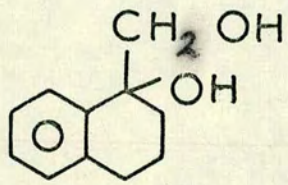

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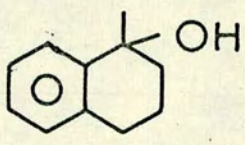


[140]

Phenylcyclohexane and derivatives.

Phenylcyclohexane [135] and its derivatives, phenylcyclohex-1-ene [107] and phenylcyclohexan-1-ol [105], on CAN oxidation, gave products which showed that an analogous reaction to that of the aromatic steroids was occurring. The major product in each case was shown to be 1-phenyl cyclohexane-1,2-diol 2-nitrate ester [136], by reduction with zinc/acetic acid to the corresponding 1-phenylcyclohexane-1,2-diol [137].

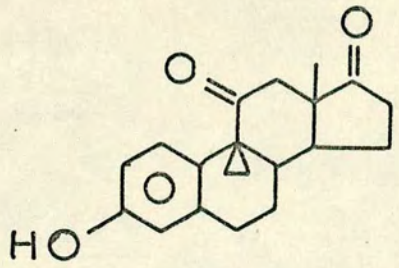
In each case a small quantity of 1-phenyl cyclohexan-1-ol was obtained showing the reaction was not as specific in mechanism as with the steroids. This was confirmed by the formation in each case of about 10% of the diol 1-phenylcyclohexan-1,2-diol by the oxidation. This suggested Ce(IV) oxidation of phenylcyclohexene, formed as an intermediate, was occurring to yield the diol directly ie cis hydroxylation. Phenylcyclohex-1-ene reacted much faster than phenylcyclohexane suggesting it was an intermediate of this reaction.

Methyl tetralin [108]

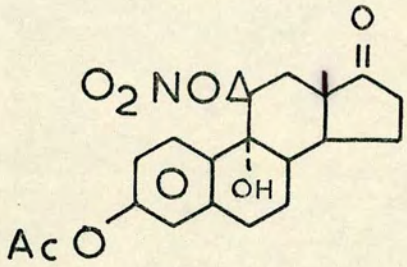
Methyl tetralin, on CAN oxidation, yielded a nitrate ester in agreement with steroid results. The N.M.R. spectrum showed that an acetate had also been formed. The products were confirmed as being 1-methyl-1hydroxytetralin 1-acetate [138], and 1-nitratomethyl-1-hydroxy tetralin [139] by I.R., N.M.R. and mass spectral data, and by conversion to the corresponding known compounds, 1-methyl-1-hydroxy tetralin [140], and 1-hydroxymethyl-1-hydroxy tetralin [150].

The results of this experiment suggest that the rigid stereochemistry of the aromatic steroids is important in the preparation of nitrate esters. Methyl tetralin has a more flexible structure than the aromatic steroids with the result that more than one pathway is available for further reaction of the intermediates formed.

The carbonium ion formed from the initial tertiary radical is



[141a]



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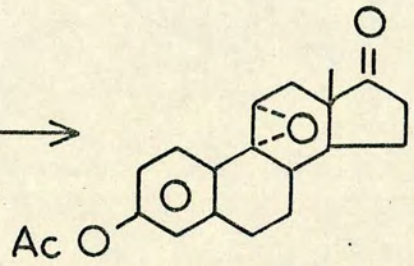

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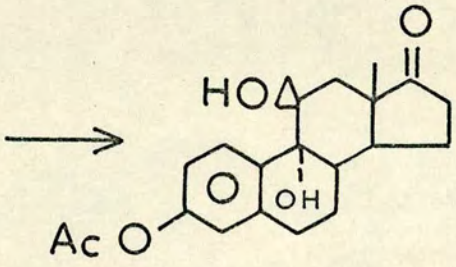
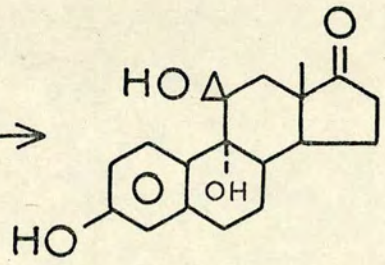


[115]



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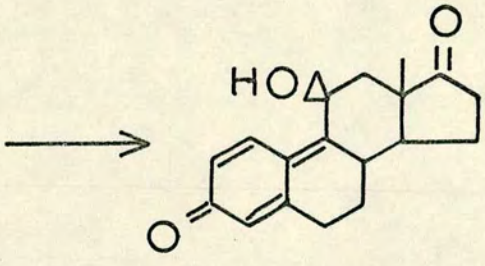

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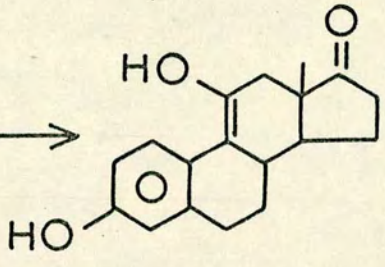

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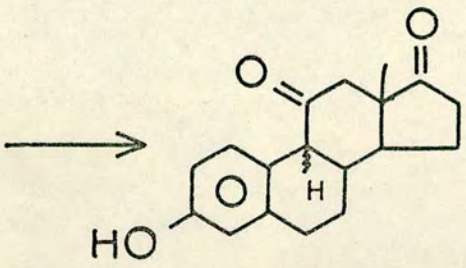


[141a] 9β-H

[141b] 9α-H

Some Reactions of the Oxidation Products of Oestrone Acetate.

These reactions may be most conveniently arranged in two sections:

1. Reactions of the 9α,11β-hydroxy nitrate derivative; and
2. Reactions of 9α,11β-dihydroxyoestrone acetate.

1. Reactions of the 9α-Hydroxy 11β-nitrate ester of Oestrone Acetate.

(a) With Base.[73,75,101]

The reactions of the hydroxy-nitrate with base run somewhat parallel to the reactions of the analogous bromohydrin[73].

With weak base, eg potassium acetate, or sodium bicarbonate[75], elimination of acid, here nitric acid, occurs to yield the known 9α,11-epoxide [72] , M.P. 160-2° (lit[73] 160-2°), which by the action of strong base[73] eg sodium carbonate or sodium hydroxide, can be converted into 3-hydroxy-9β-oestra-1,3,5(10)-triene-11,17-dione [141a] (M.P. 208-10°, lit[101] 204-7°). This latter compound can also be obtained by the direct action of strong base on the hydroxy-nitrate. The reaction of the hydroxy-nitrate with strong base is believed to occur through this same epoxy compound, which immediately reacts further to give the isolated product, the dione.

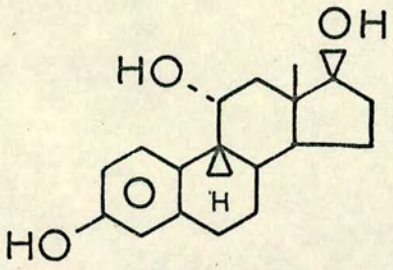
The mechanism is believed to be as outlined in the scheme opposite. Initially a basic species abstracts a hydrogen ion from the 9α-hydroxy group. Simultaneous loss of the nitrate group occurs and the 9α,11-epoxide [72] is obtained. With weak base, the reaction terminates at this stage. With stronger base, further base attack on the epoxide occurs to yield the 9α,11β-di-hydroxy derivative [115] of oestrone acetate, which however cannot be isolated because it immediately reacts further. The postulation of this 9α,11β-dihydroxy intermediate explains the fact that the 9α,11β-hydroxy nitrate gives the same product with strong base (ie the 9β-H 11-keto oestrone) as the 9α,11β-dihydroxy compound does itself.

The dihydroxy compound reacts with more base to lose its acetate group, thus generating a free phenol [142], which under the basic conditions forms the quinone methide [143]. This is confirmed by the reaction mixture turning a deep reddish colour. Loss of the 9α-hydroxy group occurs at this stage. Removal of base during the work-up regenerated the phenolic ring, with formation of the enol of the 11-ketone [144]. This then reverts to the 11-ketone itself [141b].

It was found that the method of base removal in the work-up affected the product, in the case of sodium carbonate. With the reaction of the nitrate ester [116], the base was removed by neutralisation with excess acetic acid, whereas with the reaction of the 9α,11β-dihydroxy compound [115], the base removal was effected by washing with water only. The latter yielded the normal 9α,-11,17-dione [141b] whereas the former gave the 9β-isomer [141a]. This change was believed to be due to the epimerisation of the initially formed normal 11,17-dione [141b] by the acidic conditions. This was confirmed by the conversion of the normal 11,17-dione to the 9β-isomer by the action of acid. It was found that reaction with stronger base only yielded the 9β-isomer. In these reactions, it was necessary to neutralise the base, sodium hydroxide, by acid to enable the isolation of the product.

(b) Reduction to the 9α,11β-dihydroxy derivative [115].[109,110,111,74]

This reaction may be effected by any of the following three methods. Catalytic hydrogenolysis of the nitrate ester with 10% palladium on charcoal is a clean fast method[109] for the production of 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (M.P. 198-202°). Unfortunately however the product generally gives wrong C,H analysis figures owing to the presence of colloidal carbon from the catalyst, although the T.L.C., and I.R. and N.M.R. spectra of the product confirm its structure satisfactorily. Hydrogenolysis is also only applicable to a freshly prepared sample of the hydroxynitrate. When a four-week



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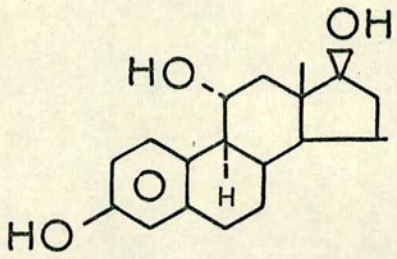
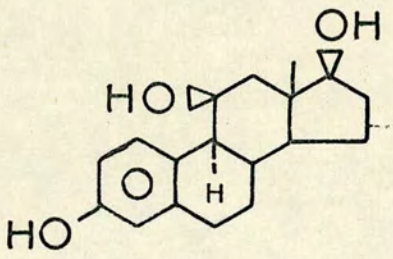


[146]



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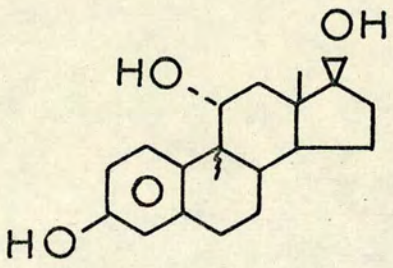

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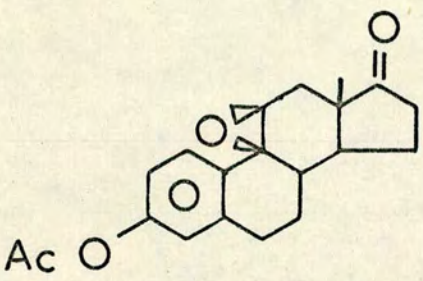

[73]

old sample of the nitrate ester was reduced, it was found that no hydrogen up-take occurred, and the compound was recovered unaffected. This was due to partial decomposition of the nitrate group to liberate nitrite ions, which have been shown capable of poisoning palladium catalysts[110]

Reduction with zinc, in acetic acid, or aqueous ethanol also reduces the 11β-nitrate group to yield the same 9α,11β-diol as was isolated by hydrogenolysis. The reaction with zinc has the advantage that it can be used with "old" samples of the hydroxy nitrate. Zinc/acid is a standard method[111] of reduction of nitrates. Reaction proceeds without change of configuration and therefore the method is ideal for this reaction. The yields of 9α,11β-diol for these two methods are comparable (60%).

Reduction with Raney nickel[74] yields the same 9α,11β-dihydroxyoestrone derivative. In this case, however, the yield was lower (40%). The product was however isolable by T.L.C., and M.P. and spectra confirmed its structure.

(c) Metal-Hydride Reduction.[73,101]

Reduction of the 9α,11β-hydroxy nitrate with either sodium borohydride or lithium aluminium hydride yielded the 3,11α,17β-trihydroxy-9β-oestra-1,3,5(10)-triene [145] M.P. 258-60° (lit[73] 254-5°). The reaction is believed to occur via a 9α,11-epoxide intermediate. The nitrate ester group, under the basic conditions used, is a good leaving group and leaves to give a 9α,11-epoxy compound. Reduction of this epoxide gives the 11α-hydroxy compound.

The mechanism of this reduction again is paralleled by the reaction of the corresponding 9,11-bromohydrin, though here the reaction proceeds in one stage. With the bromohydrin, however, the overall reduction is accomplished in two stages; first, reaction with base yields the 9α,11-epoxide, which is isolated, and this is then reduced to the 11α-hydroxy steroid.

Tsuda has reported the reduction of this 9,11-bromohydrin twice. In one report[101], he obtained approximately 50% of the 9α-oestra-1,3,5(10)-triene-3,11α,17β-triol [146], with M.P. 249-50°. In the other report[73], in two attempts he obtained 50% of 9α-oestra-1,3,5(10)-triene-3,11β,17β-triol [147], M.P. 289-91°, and later 50% of the 9α-oestratrien-3,11α,17β-triol [148], M.P. 254-55°. There would therefore appear to be some confusion as regards the product of this reduction.

On the basis of the product melting point and the known mechanism of hydride reductions, the product obtained by hydride reduction of hydroxy-nitrate was designated as being 3,11α-17β-trihydroxy-9β-oestra-1,3,5(10)-triene [145]. Hydride attack of a 9α,11-epoxide would occur at the 9β-position, to yield an 11α-hydroxy group and 9β-configuration. N.M.R. confirmation could not be obtained owing to the insolubility in deuterochloroform of the steroid product.

2. Reactions of 9α,11β-Dihydroxyoestrone Acetate [115].

(a) With Base.

These reactions are found to be similar to those of the corresponding hydroxy-nitrate. With strong base, aqueous ethanolic sodium hydroxide, 3-hydroxy-9β-oestra-1,3,5(10)-triene-11,17-dione is again obtained; (the mechanism of this reaction has already been discussed during the discussion of the reaction of the hydroxy-nitrate.) With the weaker base, aqueous ethanolic sodium carbonate, the reaction product differs from that obtained from the hydroxy-nitrate. The hydroxy-nitrate yielded the 9β-11,17-dione [141a] derivative, whereas the diol yielded 3-hydroxyoestra-1,3,5(10)triene-11,17-dione [141b]. This difference has been shown to occur due to a difference in the work-up of the reaction, and not due to the reaction itself.

This diol has already been shown to be an intermediate in the reaction of the reaction of the hydroxy-nitrate with base to yield the 11-keto-9-iso oestrone derivative, and therefore no difference in product would be expected as was indeed found.

(b) Reduction with Metal Hydride.[73]

The reduction, with sodium borohydride, yields an 11β-hydroxy substituted oestradiol derivative, the 9- and 11-isomer of the corresponding product obtained from the hydroxy-nitrate. The reaction mixture does not turn red, showing that this reaction does not proceed via the quinone methide of the base reaction. Again the reaction occurs via production of the epoxide, but in this case the 9β,11-epoxide[73]; the 9α-hydroxy group becomes the leaving group thereby alleviating the steric crowding of this tertiary position. This epoxide is then reduced by normal hydride attack at C-9 on the α-face to give the 11-hydroxy steroid, 3,11β,17β-trihydroxyoestra-1,3,5(10)-triene [147] (M.P. 290-3°, lit[73] M.P. 289-91°).

(c) Other reactions.

Reaction with Nitric Acid/Acetic Anhydride.[112]

A standard method[112] of forming nitrate esters is the reaction of alcohols with nitric acid/acetic anhydride. Treatment of the 9α,11β-diol, obtained by reduction of the hydroxy-nitrate, with this reagent returns the molecule to the same hydroxy-nitrate, showing that only the secondary C-11 hydroxyl group is easily esterified. This is in agreement with the reaction of 3,9α,11β-trihydroxy oestra-1,3,5(10)trien-17-one 3-acetate with acetic anhydride to yield only a 3,11β-diacetate, which confirms the remaining hydroxyl group, at C-9, is tertiary.

This reaction, giving the same hydroxy-nitrate shows that no structural changes occur during the reduction of the hydroxy-nitrate.

Oxidation with Jones Reagent.[64,125]

Oxidation of the 9α,11β-dihydroxy steroid with Jones reagent[64] yields the known 11-keto compound [114] (M.P. 247-8°, lit[125] M.P. 235-43°). This reaction is essential for determination of the structure of the product of CAN oxidation of oestrone acetate (see P25). The compound obtained from the Jones oxidation has a 9α-hydroxy group which confirms the stereochemistry at this position. N.M.R. figures then enable elucidation of the rest of the structure.

EXPERIMENTAL RESULTS

Melting points were determined on a Köfler block and are corrected. I.R. spectra, unless otherwise specified, were recorded for bromoform solution on a Unicam S.P.200 spectrophotemeter. N.M.R. spectra were recorded for deuterochloroform solutions using tetramethylsilane as an internal standard on a Perkin-Elmer R10 (60 MHz) N.M.R. spectrometer or a Varian HA100 (100 MHz) spectrometer. Microanalyses were carried out in this department using a Perkin Elmer Elemental Analyser, Model 240. Unless otherwise specified;

(a) Column chromatography was carried out using Fisons' Laboratory Reagent Silica Gel or Laporte type H alumina.

(b) T.L.C. was carried out using Kieselgel GF_{254} nach Stahl from Merck. Plates were prepared by thoroughly mixing 30g silica with 60g water to give a slurry. This slurry was applied using Stahl's method[113], to 20 plates, 20cms x 5 cms, to give an even layer, 250μ in thickness. The plates were dried overnight, over self-indicating silica gel, and were stored in the same way until required.

Preparative T.L.C. was carried out using plates, 20 cms x 20 cms in size, with layers approximately 1mm thick which were stored in an oven at 100°, approximately, until required.

Solvents used for T.L.C. were mixtures of ethanol:benzene (1:9) and ethyl acetate:hexane (1:9 to 1:3). After running, the plates were removed from the chromatography tank and the solvent allowed to evaporate. Plates were developed by irradiation with U.V. light (λ 254nm), and, in qualitative work only, by spraying with ethanol:sulphuric acid (9:1) followed by baking on a hot-plate at 400°C approximately. Both methods revealed the positions of the adsorbed material.

(c) Solutions were dried with magnesium sulphate.

Preparation of derivatives of Oestrone.

Oestrone Acetate.[56]

Commercial oestrone (10g, 37mM) was dissolved in pyridine (100 ml), acetic anhydride (20 ml, 0.2M) was added and the solution was left overnight at room temperature. Water (20 ml) was added, and the solution was heated on a warm water-bath for twenty minutes and, after cooling, poured into ether. The ether solution was washed with dilute hydrochloric acid, till acid to litmus, saturated sodium bicarbonate solution, till basic to litmus, and water, till neutral. The solution was dried and the solvent was evaporated to give a pale yellow syrup which crystallised on cooling. Re-crystallisation from aqueous methanol gave the product as white crystals of oestrone acetate (11g, 95%), M.P. 123-5° (lit[56] 123-4°), ν max 2950, 1750, 1725 cm^{-1}, N.M.R. absorptions at τ 2.61, 2.75, (proton on C-1), 3.10, 3.21, (protons on C-2 and C-4), 7.76, (acetate methyl), 9.11, (C-18 methyl).

3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one.[61]

Oestrone (0.5g, 1.85mM) was dissolved in methanol, (150ml) and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ, 0.45g, 2.11mM) was added. The dark-green solution was stirred at room temperature for one hour, during which time the solution changed colour, through dark red, to orange. The solvent was evaporated and the steroid was extracted into benzene, and filtered to remove the insoluble quinol formed from the DDQ. The benzene was evaporated, to leave the product as pale yellow crystals of the required product, contaminated with DDQ. The product was recrystallised from methanol to give white crystals of 3-hydroxy-oestra-1,3,5(10),9(11)-tetraen-17-one (0.38g, 76%), M.P. 250-6° (lit[61] 257-9°), ν max 3550, 2930, 1725cm^{-1}.

3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one (1g, 3.7mM), was dissolved in pyridine (5ml) and acetic anhydride (2ml, 20mM) was added. The

solution was left overnight at room temperature. The usual work-up gave the product as red crystals. (The red colouring was due to contamination by a complex formed between DDQ and pyridine.) The product was dissolved in benzene (5ml) and was refluxed for five minutes with decolourising carbon. The solution was cooled, filtered through Celite, and the solvent evaporated to give white crystals. The product was recrystallised from aqueous methanolto give 3-hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate, (1g, 90%), M.P. 125-7° (lit[57] 128-9°), ν max 2950, 1750, 1730, 1210cm^{-1}, N.M.R. absorptions at τ 2.35, 2.51, 3.11, 3.22, 3.79 (proton on C-11), 7.77, 9.11.

9β-Oestrone Acetate.

9β-Oestrone (0.1g, 0.37mM) was dissolved in pyridine (5ml) and acetic anhydride (1ml, 10mM) was added. The solution was left overnight at room temperature. The usual work-up gave a very pale brown syrup, which would not crystallise. T.L.C. showed this was pure 9β-oestrone acetate. ν max 2950, 1750, 1725cm^{-1}, N.M.R. absorptions at τ 6.4 (complex multiplet, 9β-proton), 7.76, 9.02.

Oestrone Propionate.[58]

Oestrone (1g, 3.7mM) was dissolved in pyridine (10ml) and propionic anhydride (3ml, 3g, 23mM) was added. The solution was left overnight at room temperature. The usual work-up yielded the product as white crystals which were recrystallised from ethyl acetate to give oestrone propionate (1.05g, 88%), M.P. 134-6°, (lit[58] 134-6), ν max 3650, 2950, 1730, 1380cm^{-1}, N.M.R. absorptions at τ 2.67, 2.81, 3.10, 3.17, 3.23, 7.39, 7.51, 7.63, 7.75, 8.64, 8.76, 8.88.

Oestradiol.[56]

Oestrone (1g, 3.7mM) was dissolved in peroxide-free tetrahydrofuran (THF, 50ml). Lithium aluminium hydride (1g, 26mM) was added slowly. The solution turned to a curdy grey mass, and more THF was added. The mixture

was stirred at room temperature for thirty minutes. Ethyl acetate (25ml) was added slowly to destroy the excess lithium aluminium hydride. There was a vigorous reaction. The grey curdy mixture was poured into dilute hydrochloric acid and the steroid extracted into ethyl acetate (3 x 50ml portions). The ethyl acetate solution was washed twice with dilute hydrochloric acid, twice with saturated sodium bicarbonate solution, and twice with water. The solution was dried, and the solvent evaporated to give white crystals which were recrystallised from acetone to give oestradiol (0.85g, 85%). M.P. 175-75° (lit[56] 175-8°), pure by T.L.C. and I.R. spectrum had no carbonyl peak, ν max 3550, 2930cm^{-1}.

Oestradiol Diacetate.[56]

Oestradiol (1g, 3.7mM) was dissolved in pyridine (10ml) and acetic anhydride (4ml, 40mM) was added. The solution was left overnight at room temperature. The usual work-up yielded the product as white crystals. Recrystallisation from aqueous methanol yielded oestradiol diacetate (1.0g, 88%), M.P. 126-7°, (lit[56] 125-6°), ν max 2950. 1720 (with a shoulder at 1730), 1260, 1240cm^{-1}, N.M.R. absorptions at τ 2.61, 2.79, 3.10, 3.20, 5.14, 5.25, 5.40, (α-proton on C-17) 7.73, 7.95, (acetate on C-17), 9.18.

Oestrone Benzoate.[56]

Oestrone (1g, 3.7mM) was dissolved in pyridine (10ml) and benzoyl chloride (3ml, 3.6g, 25mM) was added. The solution was left overnight at room temperature. The usual work-up gave the product as white crystals. Recrystallisation from acetone (or methanol) gave oestrone benzoate. (1.0g, 70%) M.P.214-6° (from acetone), 215-7° (from methanol) (lit[56] 217.5, from methanol), ν max 2950, 1725, 1270, 1230, 1075cm^{-1}, N.M.R. absorptions at τ 1.7-1.9 2.3-2.6, 2.9,3.1, (very complex aromatic region), 9.10.

Oestrone Methyl Ether (3-Methoxyoestrone).[56]

Oestrone (1.16g, 4.3mM) was added to a solution of methyl p-toluene sulphonate (2.3g, 12.3mM) in 10% aqueous potassium hydroxide solution (10ml) and the resulting suspension was stirred at 90° for three hours, with a further portion of alkali solution (5ml) being added after one hour. The mixture was cooled to room temperature, and the product was filtered, washed well with water, and dried in a dessicator over calcium chloride. Recrystallisation from methanol gave white crystals of oestrone methyl ether, (1.1g, 83%) M.P. 171-3° (lit[56] 167.5-169.5°), υ max 2910, 1720, 1500cm^{-1}, N.M.R. absorptions at τ 2.72, 2.87, 3.23, 3.37, 6.24, 9.12.

3-Methoxyoestra-1,3,5(10),9(11)-tetraen-17-one.[59]

1. 3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one (0.2g, 0.75mM) was dissolved in methanol (10ml) containing potassium hydroxide (0.1g). Dimethyl sulphate (0.19g, 1.50mM) was added dropwise. The solution was stirred at room temperature for one hour. Excess strong base was added to destroy the excess dimethyl sulphate, and the solution was poured into ether (100ml). The ethereal solution was washed with 10% sodium hydroxide solution and water. The solution was dried and the solvent evaporated to give the product. T.L.C. showed some unreacted starting material was present. The product was dissolved in benzene and chromatographed on a short alumina column. Elution with benzene:chloroform (1:1) gave the product as white crystals. Recrystallisation from ethanol/ethyl acetate gave 3-methoxyoestra-1,3,5(10),9(11)-tetraen-17-one, (0.104g, 50%) M.P. 136-139°, (lit[59] 143-5°), υ max 2930, 1730cm^{-1}, N.M.R. absorptions at τ 2.43, 2.57, 3.24, 3.41, 3.89, 9.07.

2. 3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one (0.2g, 0.75mM) was added to a solution of p-methyl toluene sulphonate (0.5g, 2.7mM) in 10% potassium hydroxide solution (5ml). The suspension was stirred at

90° for three hours, with the addition of a further portion (4ml) of potassium hydroxide solution after one hour. The solution was cooled, and diluted with ether (100ml). The ethereal solution was washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water. The solution was dried, and the solvent evaporated to give the product as white crystals. Recrystallisation from ethanol/ethyl acetate gave 3-methoxyoestra-1,3,5(10),9(11)-tetraen-17-one (0.125g, 60%) M.P. 136-40° (lit[59] 143-5°), confirmed by I.R. and N.M.R. spectra to be identical to the compound prepared above. (An attempt to prepare this compound, using diazomethane, resulted in a mixture of products, including the required compound, and a product with the N.M.R. spectrum, characteristic of an epoxide, τ 5.93. The compound was believed to be the result of addition of diazomethane across the C-17 carbonyl group, but the compound was not investigated further.)

Oestrone Benzyl Ether (3-Benzyloxyoestrone),[60]

Oestrone (0.7g, 2.5mM) was dissolved in ethanol (60ml). Benzyl chloride (0.6ml, 0.66g, 5.2mM) and potassium carbonate (1.4g) were added and the solution was refluxed for seven hours. The solvent was evaporated and the residue taken into methylene chloride, washed with water (3 times) and dried. Evaporation of the solvent gave the product as a brown syrup which crystallised on cooling. Recrystallisation from acetone gave the product. Recrystallisation from acetone gave white crystals of 3-benzyloxyoestrone (0.8g, 87%), M.P. 128-30°, (lit[60] 129-30°) T.L.C. indicated some starting material was still present. The product was chromatographed on a short alumina column. Elution with benzene gave the product. Recrystallisation from acetone gave 3-benzyloxyoestra (0.7g, 76%) M.P. 129-30° (lit[60] 129-30°) ν max 2950, 1745cm^{-1}, N.M.R. absorptions at τ 2.65, 2.66, 3.19, 3.29, 5.01, 9.11.

Oestrone o-Nitrobenzoate.

Oestrone (1g, 3.7mM) was dissolved in pyridine (10ml) and o-nitrobenzoyl chloride (3g, 16mM) was added. The solution was left overnight at room temperature. The usual work-up gave pale yellow crystals. Recrystallisation from acetone gave oestrone o-nitro benzoate (1.1g, 70%), M.P. 193.5-5°, (Found C71.85%, H5.87%, N3.16%, $C_{25}H_{25}NO_5$ requires C71.59%, H5.96%, N3.34%), ν max 2930, 1730, 1540, 1500, 1260cm^{-1} N.M.R. absorptions at τ 1.9-2.4, 2.57, 2.73, 2.93, 3.02 (very complex aromatic region), 9.10.

Oestrone Tosylate.

Oestrone (1g, 3.7mM) was dissolved in pyridine (10ml) and p-toluene sulphonyl chloride (3g, 14.7mM) was added. The solution was kept at room temperature for three days. The mixture was poured into water (40ml) to give an oil which crystallised on standing. The crystals were filetered off and recrystallised from methanol to give oestrone tosylate (0.8g, 70%), M.P. 139-41° (Found C70.90%, H6.68%, $C_{25}H_{28}SO_4$ requires C70.75%, H6.60%), ν max 2930, 1725, 1600, 1385, 1220cm^{-1}, N.M.R. absorptions at τ 2.21, 2.34, 2.78, 2.91, (tosylate peaks) 2.63, 3.25, 3.40, 7.57, 9.12.

3-Desoxyoestradiol (17β-Hydroxyoestra-1,3,5(10)-triene).[62,63]

Oestrone, (1g, 3.7mM) was dissolved in THF (16ml) and added to a solution of tri-ethylamine (4.0ml, 2.9g, 29mM), and diethyl phosphite (6.0ml, 6.2g, 38mM) in carbon tetrachloride (30ml). The solution very quickly became warm and turned pink. The solution was kept at room temperature for 72 hours. The precipitate was filtered off and the solution was washed with dilute hydrochloric acid, dilute sodium hydroxide solution, and water. The solution was dried and the solvent evaporated to give a brown oil. T.L.C. indicated the desired product had been formed but streaking of the plate indicated the product was contaminated. The crude product was used in the reduction step.

The steroid was dissolved in ether (20ml) and liquid ammonia (50ml) was added. Freshly cut pieces of lithium (0.5g, 70mM) were added till the solution remained blue. The solution was stirred for one hour, and the ammonia was then allowed to boil off. Ether (100ml) was added and the solution was washed with dilute hydrochloric acid, 10% sodium hydroxide solution and water. The solution was dried and the solvent evaporated to give the product as a pale yellow oil which crystallised on cooling. Recrystallisation from methylene chloride / hexane gave white crystals of 3-desoxyoestradiol, (17β-hydroxyoestra-1,3,5(10)-triene, 0.8g, 94%) M.P. 108-110° (lit[62] 109-110°) ν max 3000,2920cm^{-1}, N.M.R. absorptions at τ 2.79, 2.82, 2.90, 2.98, 6.1, 6.3, 6.5, 9.25.

3-Desoxyoestrone (Oestra-1,3,5(10)-triene-17-one).[63,64]

1. 3-Desoxyoestradiol (0.6g, 2.3mM) was dissolved in pyridine (5ml) and added to chromium trioxide (0.8g, 8mM) in pyridine (20ml). The solution was stirred at room temperature for three hours. The solution was poured into ethyl acetate (100ml) and methanol (10ml) was added to destroy excess chromium trioxide. The ethyl acetate solution was washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water. The solution was dried and the solvent evaporated to give the product as pale yellow crystals. Recrystallisation from methanol gave 3-desoxyoestrone (0.3g, 50%), M.P. 140-1° (lit[62] 135-6°), ν max 2900, 1720cm^{-1}, N.M.R. absorptions at τ 2.78, 2.86, 2.94, 9.11. T.L.C. indicated the compound was pure.

2. 3-Desoxyoestradiol (0.2g, 0.8mM) was dissolved in acetone (2ml) and 0.23ml (5% excess) of 8N chromium trioxide in 8N H_2SO_4 was added dropwise with stirring[64]. The solution was stirred for ten minutes at room temperature. Methanol (2ml) was added to destroy excess Jones reagent. The solution was poured into ethyl acetate (50ml) and washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water. The

solution was dried and the solvent evaporated to give the product as a pale yellow syrup which was recrystallised from methanol to give 3-desoxyoestrone, identical with the sample prepared above.

2,4-Di-nitrooestrone.[66]

Oestrone (1g, 3.7mM) was dissolved in acetic acid (30ml) with warming. Complete solution was found to be impossible. Concentrated nitric acid (0.47ml, 7.4mm) was added dropwise. During the addition of nitric acid, the acetic acid solution became warm, and the oestrone dissolved. The solution was stirred overnight at room temperature. The solution was poured into ether, and washed several times with water. The solvent was evaporated, and the product was recrystallised from aqueous acetic acid to give yellow crystals of 2,4-dinitrooestrone, M.P. 185-6 (lit[66] 187-8.5°).

The mother liquor from the recrystallisation was poured into water and the yellow precipitate, which formed, was filtered off, washed with water, and recrystallised from aqueous ethanol to give a yellow amorphous material. Attempts to take the melting point of this material showed that at 180°, the amorphous material changed to crystals which melted at 185-7°.

I.R. and N.M.R. spectra of the two samples showed they were identical, ν max 3650, 2950, 1727, 1580, 1547, 1480cm^{-1}, τ 1.84, 9.08.

The two samples were combined and recrystallised from aqueous acetic acid to give 2,4-dinitrooestrone (0.7g, 54%) M.P. 186-7° (lit[66] 187-8.5°) (I.R. and N.M.R. figures as listed above.)

2,4-Dinitrooestrone Acetate (3-Hydroxy-2,4-dinitrooestra-1,3,5(10)-trien-17-one 3-Acetate),[66]

2,4-Dinitrooestrone (0.2g, 0.55mM) was dissolved in pyridine (5ml) and acetic anhydride (1ml, 10mM) was added. The solution was left overnight at room temperature. The usual work-up gave the product as pale yellow

crystals. Recrystallisation from aqueous methanol gave 2,4-dinitro-oestrone acetate (0.16g, 73%) M.P. 186-8° (lit[66] 187-8.5°), mixed M.P. with dinitro oestrone 150-60°, ν max 2950, 1780, 1725, 1545, 1380cm^{-1}, N.M.R. absorptions at τ 1.77, (proton on C-1) 7.65, 9.08.

2,4 Dinitrooestrone 3-Methyl Ether (3-Methoxy-2,4-dinitrooestra-1,3,5(10)-trien-17-one).

2,4-Dinitrooestrone (0.20g, 0.55mM) was dissolved in ether (50ml). An ethereal solution of diazomethane (50mls of 0.05M, 2.5mM) was added and the solution was stirred for five minutes. Acetic acid (5ml) was added to destroy excess diazomethane. The ether solution was washed with dilute sodium hydroxide solution till the ether layer was colourless (to remove unreacted steroid), and water, dried and evaporated to dryness.

The product would not crystallise, owing to considerable degradation during the reaction. Preparative T.L.C. on silica gave 0.135g of the desired product which was recrystallised from methylene chloride/hexane to give the pure compound, 3-methoxy-2,4,-dinitrooestra-1,3,5(10)trien-17-one (0.12g, 60%) M.P. 119-121° (Found C61.24%, H5.79% N7.43%, $C_{19}H_{22}N_2O_6$ requires C60.96%, H5.88%, N7.48%). ν max 2950, 1727, 1547cm^{-1}, N.M.R. absorptions at τ 2.01, 6.08, 9.08.

17-Desoxyoestrone (3-Hydroxyoestra-1,3,5(10)-triene).[68]

Oestrone (2g, 7.4mM), sodium hydroxide (1g), digol (16ml) and 85% hydrazine hydrate (1ml) were refluxed for one hour. The condenser was removed and the temperature was allowed to rise to 195-200°, and refluxing was continued for a further three hours. The mixture was cooled which caused the product to crystallise. Water (50ml) was added, the aqueous layer was acidified and extracted three times with 100ml portions of ether. The ether solution was washed with saturated sodium bicarbonate solution, and water, and dried. Evaporation of the solvent gave pale brown crystals. Recrystallisation from methanol gave white

crystals of 17-desoxyoestrone (1.65g 87%) M.P. 138-41° (lit[68] 140-1°) ν max 3550, 2930cm^{-1}. (No carbonyl peak.)

17-Desoxyoestrone Acetate (3-Hydroxyoestra-1,3,5(10)-triene 3-acetate).

17-Desoxyoestrone (1.1g, 4.3mM) was dissolved in pyridine (20ml) and acetic anhydride (2ml, 20mM) was added. The solution was left overnight at room temperature and worked up in the usual manner to give the product as white crystals. Recrystallisation from aqueous methanol gave 17-desoxyoestrone acetate (1.1g, 80%), M.P. 84-6° (Found C80.18%, H8.77% $C_{20}H_{26}O_2$ requires C80.57%, H8.72%), ν max 2940, 1745, 1220cm^{-1}, N.M.R. absorptions at τ 2.64, 2.79, 3.13, 3.23, 7.76, 9.27.

3-Hydroxy-19-norcholesta-1,3,5(10)-triene 3-Acetate.[69]

Cholesta-1,4-dien-3-one.[114]

Cholest-4-en-3-one (3.18g, 8.3mM) was dissolved in dioxan (100 ml). DDQ (2.071g, 9.1mM) was added and the solution was refluxed overnight. The solution was cooled to room temperature and the quinol from the DDQ was filtered off. The red solution was evaporated to dryness and the resulting red syrup was dissolved in benzene and chromatographed on alumina. Elution with benzene yielded a smallamount of unreacted DDQ and then the product, cholesta-1,4-dien-3-one which was recrystallised from acetone to give pale yellow needles of cholesta-1,4-dien-3-one (3.01g, 95%) MP 112-4° (lit[114] MP 112°) ν max 2950, 1660, 1620 cm^{-1}. N.M.R. absorptions at τ 2.76, 2.90, 3.06, 3.74, 3.92, 4.31, 8.79, 8.82, 9.10, 9.20, 9.29.

Aromatisation to 3-Hydroxy-19-norcholesta-1,3,5(10)-triene.[69]

Biphenyl (10.8g, 70mM) and freshly extruded lithium wire (0.5g, 70mM) were refluxed under nitrogen in peroxide free T.H.F. (50ml) for one hour with stirring. The resulting dark-green solution was cooled to room temperature, and cholesta-1,4,-dien-3-one (3.0g, 7.8mM) in THF (25ml) was added, and the mixture was refluxed under nitrogen with stirring for a

further hour. The solution was cooled, during which it turned blue, filtered to remove lithium, evaporated to dryness and the residue was taken into ether. The ethereal solution was washed twice with dilute hydrochloric acid (to remove lithium compounds) which caused the colour to change to pale brown, and with water (5 times), dried and evaporated to dryness to give a dark brown syrup which crystallised on cooling due to the presence of the large quantity of biphenyl.

This material was steam distilled till no more biphenyl came over. The residue was taken into ether, washed twice with water and dried. The ether was evaporated tillthe solution volume was less than 50 ml, and this was extracted with 10% sodium hydroxide solution (3 x 50ml). The basic fractions were combined, and acidified cautiously with concentrated hydrochloric acid, and extracted into chloroform. The chloroform solution was washed with saturated sodium bicarbonate solution and water, dried and evaporated to dryness. T.L.C. of the residue gave one spot, with Rf typical for a steroid phenol (approximately 0.5). The product was recrystallised from methanol to give 3-hydroxy-19-norcholesta-1,3,5(10)-triene (0.4g, 13%) MP 112-4°, (lit[69] MP 118°) ν max 3570, 2920, 1600 cm^{-1}, N.M.R. absorptions at τ 2.79, 3.49, 8.76, 9.09, 9.18, 9.32 (confirming loss of C-19 methyl.)

3-Hydroxy-19-norcholesta-1,3,5(10)-triene 3-Acetate.[70]

Cholestatrienol (0.4g, 1.0mM) was dissolved in pyridine (5ml) and acetic anhydride (1ml, 10mM) was added. The solution was left overnight at room temperature. The usual work-up gave a brown syrup which crystallised on cooling. Recrystallisation from ether/methanol gave 3-hydroxy-19-norcholesta-1,3,5(10)-triene 3-acetate (0.4g, 90%), M.P. 91-3° (lit[70] MP 93.5 - 95°) ν max 2950, 1720 cm^{-1}, N.M.R. absorptions at τ 2.64, 2.75, 3.10, 3.21, 7.76, 9.29.

3-Phenylandrost-2-en-17β-ol.

Phenyl magnesium bromide.[71]

A mixture of bromobenzene (2g, 1.3ml, 12.7mM) and ether (5ml) was added to a flask containing clean magnesium turnings (1.8g, 74.1 mM) under ether (20ml). The flask was warmed gently using a water bath until the reaction began, shown by the development of cloudiness, and spontaneous refluxing. A mixture of bromobenzene (11g, 7.3ml, 70.1mM) in ether (25ml) was run in at such a rateas to cause vigorous refluxing. The mixture was stirred till refluxing stopped (1 hour).

3-Phenylandrost-2-en-17β-ol.[72]

Androstan-17β-ol-3-one (2g, 6.9mM) was dissolved in ether (200ml) and the solution was added dropwise to the phenyl magnesium bromide (approximately 70mM).

As the steroid was added, a precipitate of insoluble product was deposited. The reaction mixture was stirred for one hour at room temperature and then poured carefully into excess ice/dilute hydrochloric acid.

Ether (300 ml) was added and the ether solution was washed with dilute hydrochloric acid, dilute sodium hydroxide solution (to remove phenol formed), and water. The solution was dried, and the solvent evaporated to give a pale brown syrup. Vacuum distillation removed excess bromobenzene to give pale yellow crystals. T.L.C. of the product showed biphenyl had been formed[72]. T.L.C. also showed the reaction gave two products which were believed to be the 3-hydroxy compound (Rf 0·6, blue spot) and the required product (Rf 0.75, blue spot.) The crude product was dissolved in acetic acid (100 ml) and anhydrous hydrogen chloride (2g) was added. The solution was refluxed for thirty minutes. The solution was cooled to room temperature and the acid was neutralised by the addition of solid sodium bicarbonate. The steroid

was extracted into ether, washed with water, and dried. Evaporation of the solvent gave pale brown crystals as product. T.L.C. showed this consisted of starting material androstan-17β-ol-3-one (Rf 0.7, brown spot), 3-phenylandrost-2-en-17β-ol (Rf 0.75, blue spot) and biphenyl (Rf 0.9). The product was dissolved in benzene and chromatographed on silica. Elution with benzene:petrol (1:4) mixture gave biphenyl. Elution with benzene gave the two steroidal compounds as one fraction.

The steroid mixture (1g) was dissolved in ethanol (20ml) and sodium borohydride (1g) was added. The solution was stirred at room temperature for one hour. Acetic acid (5ml) was added to destroy the excess sodium borohydride, and the solution was poured into ether (100ml). The solution was washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water, and dried. Evaporation of the solvent gave the product as pale brown crystals. The product was dissolved in benzene and chromatographed on alumina. Elution with benzene:chloroform (1:1) gave the required product as white crystals. Recrystallisation from acetone gave white needles of 3-phenylandrost-2-en-17βol M.P. 138-9°, (Found C85.39%, H9.85%, $C_{25}H_{34}O$ requires C85.71% H9.71%) ν max 3550, 2930, cm^{-1}, N.M.R. absorptions at τ 3.6-3.9, 6.2-6.4, 9.21, 9.26 (Poor solubility in $CDCl_3$ resulted in small NMR peaks.)

Acetylation of product.

The product from the previous reaction (0.6g, 0.17mM) was dissolved in pyridine (5ml) and acetic anhydride (1ml, 10mM) was added. The solution was left overnight at room temperature. The usual work-up gave the product as white crystals. Recrystallisation from acetone gave the product, M.P. 168-70°, ν max 2920, 1717, cm^{-1}, N.M.R. absorptions at τ 2.5-2.9, 3.9-4.0, 4.26, 5.34, 5.43, 5.51, 8.00, 9.22, 9.25. Examination of the NMR spectrum showed the product consisted of a mixture of two compounds; 3-phenyl-androst-2-en-17β-ol 17β-acetate, and

3-phenylandrost-3-en-17β-ol 17β-acetate. Examination of the signals at τ3.9-4.0 and 4.26, showed the major product (85%) was 3-phenylandrost-2-enyl acetate. The peak shape at τ3.9-4.0 was a complex triplet, indicating the olefinic proton was adjacent to two protons and therefore to C-1. The peak shape at τ4.26 was a complex doublet indicating the olefinic proton was adjacent to one proton and therefore to C-5. The yield of product was 0.6g, (88% for the acetylation step, 22% calculated on androstanolone), and the mixture analysed correctly for the molecular formula. (Found: C84.67%, 9.28%, $C_{27}H_{36}O_2$ requires C84.65%, H9.18%).

Preparation of Compounds for Investigation of the Mechanism of the Oestrone Acetate/CAN Reaction.

3,9α-Dihydroxyoestra-1,3,5(10)-trien-17-one 3-Acetate;[73]

11β-Bromo-3,9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-Acetate[73]

3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate (2g, 6.5mM) was dissolved in acetone (50ml) and N-bromosuccinimide (1.6g, 9.1mM) was added. The solution was cooled to 0° in an ice-bath. Perchloric acid (10.5mls of 0.2N) was added dropwise over fifteen minutes and the solution was left for a further thirty minutes. 10% Sodium thiosulphate solution was added dropwise tillthe solution failed to give a positive test with moist starch/iodide paper. The solution was poured into ether (150ml) and washed with water. The solution was dried and the solvent evaporated to give a brown oil which crystallised on standing. The product was recrystallised from ether to give 11β-bromo-3,9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (0.8g, 30%), M.P. 112-4° (with decomposition), (lit[73] M.P. 112-3°), υ max 2930, 1740, 1705, 1610cm^{-1}, N.M.R. absorptions at τ 2.50, 2.64, 2.80, 3.02, 3.16, 5.14, 7.74, 8.76.

Debromination by Raney Nickel.[74, 73]

11β-Bromo-3,9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (0.53g, 1.30mM) was dissolved in ether (14 ml) and Raney nickel (10g)[74] in dioxan (20ml) was added. The solution was stirred for eight hours in the dark. The nickel was carefully filtered off and the solvent was evaporated, below 50°. The product was recrystallised twice from acetone/hexane to give white crystals of 3,9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (0.36g, 84%), M.P. 166-8° (lit[73] M.P. 167-8°), υ max 3600, 2930, 1735, 1720, 1610cm^{-1}, N.M.R. absorptions at τ 2.42, 2.57, 3.17, 7.76, 9.12.

3-Hydroxy-9α,11-epoxyoestra-1,3,5(10)-trien-17-one 3-Acetate.[73]

3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate (10g, 3.2mM)

was dissolved in chloroform (10ml), m-chloroperbenzoic acid (0.55g, 3.8mM) was added and the solution was stirred for one hour. The excess m-chloroperbenzoic acid was neutralised by the addition of sodium thiosulphate (1g, in 5mls water). The solution volume was increased to 50mls by the addition of chloroform and the solution was washed with saturated sodium bicarbonate solution, and water and dried. The solvent was evaporated and the product recrystallised from acetone to 3-hydroxy-9α,11-epoxyoestra-1,3,5(10)-trien-17-one 3-acetate (0.6g, 57%) M.P. 160-2°, (lit[73] M.P. 160-2°), υ max 2930, 1740, 1705, 1610cm^{-1}, N.M.R. absorptions at τ 2.50, 2.64, 2.80, 3.02, 3.16, 5.14, 7.74, 8.76.

Attempted preparation of 3-Hydroxy-9β,11-epoxyoestra-1,3,5(10)-trien-17-one 3-Acetate.[75]

1. 3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate (0.5g 1.6mM) was dissolved in dioxan (25ml) and water (2.5ml) was added. N-Bromo-succinimide (0.3g, 1.7mM) and 1.5N perchloric acid (2ml) were added. The solution was stirred at room temperature for three hours in the dark. The solution was poured into ether and the ether solution was washed with saturated sodium bicarbonate solution and water, and dried. The solvent was evaporated at low temperature, (below 40°), to give a pale-brown syrup which was recrystallised from ether to give 11β-bromo-3,9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (0.2g, 30%) M.P. 112-4° (with decomposition), (lit[73] M.P. 112-3°), υ max 2930, 1740, 1705, 1610cm^{-1}, N.M.R. absorptions at τ 2.50, 2.64, 2.80, 3.02, 3.16, 5.14, 7.74, 8.76. (Note: Literature[75] reports this route to the 9α-bromo 11β-hydroxyanalogue, prior to dehydrobromination to 9β,11 -epoxide.)

Dehydrobromination to Epoxy Oestrone Acetate.[73]

3,9α-Dihydroxy-11β-bromooestra-1,3,5(10)-trien-17-one 3-acetate (0.15g, 0.37mM) was dissolved in acetone (10ml). Potassium acetate (0.3g, 0.75mM) was added and the solution was refluxed overnight. The

solution was cooled, poured into ether, washed with water, and dried. The solvent was evaporated to give the product as crystals. Recrystallisation from ether gave 3-hydroxy-9α,11-epoxy oestra-1,3,5(10)-trien-17-one 3-acetate (72mgs, 60%) M.P. 159-61° (lit[73] M.P. 160-2°) N.M.R. absorptions at τ 5.90, 7.76, 9.11.

The I.R. spectrum and T.L.C. confirmed this compound was identical with that prepared by the reaction between 3-hydroxyoestra-1,3,5(10), 9(11)-tetraen-17-one 3-acetate and m-chloroperbenzoic acid.

Epimerisation of 9α,11-epoxide group.

2. 3-Hydroxy-9α,11-epoxyoestra-1,3,5(10)-trien-17-one 3-acetate (0.1g, 0.31mM) was dissolved in acetone (5ml) and potassium acetate (0.1g, 0.25mM) was added. The solution was refluxed overnight . The solution was poured into ether, and the ether solution was washed with water, and dried. Evaporation of the solvent gave the product which was recrystallised from ether to give 3-hydroxy-9α,11-epoxyoestra-1,3,5(10)-trien-17-one 3-acetate (0.09g, 90%) M.P. 160-2° (lit[73] M.P. 160-2°), confirmed by I.R. and N.M.R. spectra and T.L.C. to be identical with the starting material.

Using Peracetic Acid.

3. Peracetic acid was prepared by the reaction of acetic acid with hydrogen peroxide. This solution has been reported[108] to contain 2-2.5% peracetic acid.

3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate (0.1g, 0.32mM) was dissolved in acetic acid (2ml) and the peracetic acid solution (8mls, a ten-fold excess) described above was added. The solution was stirred at room temperature for fifteen minutes, and then the excess oxidant was neutralised with excess 10% sodium thiosulphate solution. The solution was poured into ethyl acetate and the ethyl acetate solution was washed with saturated sodium bicarbonate, and water, and dried. Evaporation of

the solvent yielded a product (95mgs, 95%) which was shown by T.L.C., and N.M.R., and I.R. spectra to consist only of unreacted starting material, showing no epoxide had been formed.

Attempted Preparation of 3-Hydroxy-9α-Fluorooestra-1,3,5(10)-trien-17-one 3-Acetate.

1. 3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate (1g, 3.2mM) was dissolved in pyridine (10ml) and anhydrous liquid hydrogen fluoride (5ml) was added dropwise. There was a very violent reaction between the pyridine and the hydrogen fluoride. The solution was stirred overnight at room temperature. The solution was poured into ether (100ml) and washed with saturated sodium bicarbonate solution (to remove hydrogen fluoride), dilute hydrochloric acid (to remove pyridine), saturated sodium bicarbonate solution (to remove acid), and water. The solution was dried and the solvent evaporated to give a pale yellow oil, which crystallised on standing. T.L.C. suggested the product consisted of hydrolysed starting material. The product was acetylated in the usual manner to give 3-hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate, M.P. 124-6° (lit[57] M.P. 128-9°). The massspectrum had no peak at m/e = 19, indicating no incorporation of fluorine, and the parent ion had m/e = 310, again showing the product was unchanged starting material(Mol. Wt.310).

The reaction was repeated using acetic acid (10ml) as solvent, overnight at room temperature, and methylene chloride (10ml) as solvent, three hours at room temperature. In each case, there was 95% return of starting material.

2. 3,9α-Dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (20mgs, 0.061mM) was dissolved in pyridine (5ml) and anhydrous hydrogen fluoride (5ml) was added dropwise. There was a very violent reaction between the pyridine and the hydrogen fluoride. The solution was stirred overnight at room temperature. The solution was poured in ether (100ml) and worked

up as with the previous attempt with pyridine as solvent. A pale yellow oil which crystallised on standing was obtained as product. T.L.C. suggested the product was 3-hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one. The product was acetylated in the usual manner to give 3-hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate (from N.M.R. figures). The mass spectrum had no peak at m/e = 19, indicating no incorporation of fluorine and the parent peak had m/e = 310, corresponding to 3-hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate, with a smallpeak at m/e = 328, corresponding to the original starting material, 3,9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate.

The reaction was repeated with methylene chloride as solvent. 3,9α-Dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (20mgs, 0.061mM) was dissolved in methylene chloride (10ml) and anhydrous hydrogen fluoride (5ml) was added dropwise. The solution was left overnight at room temperature. The usual work-up yielded the product as a pale yellow oil. The product was acetylated in the usual manner to give the product. The mass spectrum had a small peak at m/e = 19, and this showed that a small amount of fluorine had been incorporated. Comparison of the peak heights of the parent ions of the starting material, m/e = 310, 3-hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate, m/e = 310, and 9α-fluoro-3-hydroxyoestra-1,3,5(10)-trien-17-one 3-acetate, m/e = 330 suggested the yield was less than 5%. Comparison of the N.M.R. peak heights at τ 3.79, and τ 9.11 also showed that only a very small yield of the required product had been obtained.

The preparation was not investigated further.

<u>3-Hydroxy-9β-oestra-1,3,5(10)-trien-11,17-dione.</u>[101]

3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.26g, 0.67mM) was dissolved in methanol (30ml) and sodium hydroxide (0.2g) was added. The solution changed colour from pale yellow to deep

red-purple as the sodium hydroxide dissolved. The solution was refluxed for one hour, cooled to room temperature and acidified with acetic acid. This caused the colour to change to reddish-brown. The solution was poured into chloroform (100ml) and washed with saturated sodium bicarbonate solution, and water. The solution was dried and the solvent evaporated to give a crystalline product. Recrystallisation from ether gave 3-hydroxy-9β-oestra-1,3,5(10)-trien-11,17-dione, (0.072g, 40%) M.P. 205-10° (lit[101] M.P. 204-7°), ν max 3550, 2920, 1730, 1695, 1250cm^{-1}, N.M.R. absorptions at τ 3.16, 3.37, 6.3 (complex multiplet 9β-hydrogen), 9.08.

3-Hydroxy-9β-oestra-1,3,5(10)-triene-11,17-dione 3-Acetate.

3-Hydroxy-9β-oestra-1,3,5(10)-triene-11,17-dione (0.05g, 0.17mM) was dissolved in pyridine (5ml) and acetic anhydride (1ml, 10mM) was added. The solution was left overnight at room temperature. The usual work-up gave a pale-brown syrup which would not crystallise. Spectral evidence identified this as 3-hydroxy-9β-oestra-1,3,5(10)-triene-11,17-dione 3-acetate, (57mgs, 97%), ν max 2920, 1740, 1695, 1250cm^{-1}, N.M.R. absorptions at τ 6.3, 7.75, 9.08. T.L.C. confirmed the compound was pure.

Preparation of Non-Aromatic Steroids.

The cholesterol, cholest-4-enone and cholest-5-enone used were commercial samples. T.L.C. was used to check their purity. All were found pure enough to use without further purification.

Allocholesterol (Δ 4-Cholesterol).[78,79,80,115]

Lithium aluminium hydride (0.75g, 18.7mM) was added to dry ether (70mls) and the suspension was stirred. The temperature was maintained at room temperature while cholest-4-enone (2.5g, 6.5mM) in dry ether (55ml) was added dropwise. The flask contents turned to a curdy grey mass during the addition of the steroid. The mixture was heated under reflux for thirty minutes, cooled to room temperature and the excess lithium aluminium hydride was destroyed by the cautious addition of water (25ml). The ether solution was washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water. Drying of the solution and evaporation of the solvent gave white crystals as product. T.L.C. showed the product consisted of an approximately 9:1 mixture of allocholesterol, and 3α-cholestanol. The product was dissolved in benzene and chromatographed on alumina. Elution with 5% chloroform in benzene gave the following crystalline fractions.

1. 3α-cholestanol (0.09g, 3.6%) M.P. 140-1° (lit[115] M.P. 141-2°) ν max 3570, 2920, 1470, cm^{-1}, N.M.R. absorptions at τ 5.75, 5.88, 5.99, 8.24, 8.97, 9.20, 9.34.

2. Allocholesterol (1.4g, 56%) M.P. 132-3° (lit[80] M.P. 132°), ν max 3570, 2920, 1650, 1470cm^{-1}, N.M.R. absorptions at τ 4.70, 5.72, 5.85, 5.97, 8.24, 8.97, 9.11, 9.20, 9.34.

Recrystallisation from methanol gave allocholesterol M.P. 132-3° (lit[80] M.P. 132°).

Lithium Aluminium Tri-t-Butoxide Hydride.[78]

t-Butanol (3ml, 24g, 32.0mM) was added slowly to a suspension of

lithium aluminium hydride (0.4g, 10mM) in THF (30ml) at 0°. The suspension was stirred for fifteen minutes and used without further treatment in the next step.

Allocholesterol.

Cholest-4-enone (1g, 2.6mM) was added to the reagent prepared above, and stirred for thirty minutes at 0°, and then room temperature for one hour. The mixture was then poured into excess dilute sulphuric acid, extracted with ether (3 x 50ml), washed with water till neutral, dried and evaporated to dryness to give white crystals. T.L.C. of this material showed it consisted of two components allocholesterol (cholest-4-en-3β-ol), and a small amount of cholest-4-en-3α-ol. The product was recrystallised from acetone to give allocholesterol (0.80g, 80%) M.P. 130-2°, (lit[80] M.P. 132°), ν max 3570, 2920, 1650, 1470, 1390cm^{-1}, N.M.R. absorptions at τ 4.70, 5.72, 5.85, 5.97, 8.24, 8.97, 9.11, 9.20, 9.34.

Purification by Bromination.[79]

Impure allocholesterol (2g, 5.2mM) was dissolved in ether (20ml), with warming and stirring, and the solution was cooled to room temperature. Sodium acetate (0.070g) was dissolved in acetic acid (8ml) with warming and stirring to act as a buffer for the bromination. Bromine (0.9g, 0.3ml, 5.6mM) was added to the acetic acid solution, and this mixture was added to the ether solution. The solution turned a deep-red colour, and faded quickly to pale yellow. Nitrogen was blown through the solution to remove the ether. The product precipitated, and the precipitate was filtered off and washed with acetic acid tillcolourless. A sample of the product was recrystallised from ethyl acetate/methanol to give white crystals of 4,5 dibromo-cholestan-3β-ol, M.P. 129-31° (Found C59.63% H8.47%, $C_{27}H_{46}Br_2O$ requires C59.34%, H8.42%) N.M.R. absorptions at τ 4.87, 5.48, 8.63, 9.09.

Dibromocholestanol (60mgs, 0.11mM) was dissolved in ether (25ml)

and excess zinc dust (0.3g) was added[79]. The mixture was stirred at room temperature for twenty minutes with the addition of portions of zinc dust (0.1g) every five minutes. Pyridine (5ml) was added and the solution was filtered. Ether (50ml) was added and the solution was washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water, and dried. Evaporation of the solvent gave the product, which was recrystallised from acetone to give allo-cholesterol M.P. 132-3° (lit[80] M.P. 132°), confirmed by N.M.R. spectra. When the debromination reaction was repeated on a larger scale, allocholesterol was obtained in 75% yield, calculated on the impure starting material.

11α-Hydroxypregn-4-en-3,20-dione 11α-tosylate.
(11α-Hydroxyprogesterone 11α-Tosylate).[116]

11α-Hydroxyprogesterone (2g, 6.1mM) was dissolved in pyridine (20ml) and p-toluene sulphonyl chloride (2g, 9.7mM) was added. The solution was left at room temperature for three days, during which time the solution turned a pink colour. The solution was poured into excess ice/water and extracted into chloroform. The chloroform solution was washed till neutral with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water. The solution was dried and the solvent evaporated to give a golden-brown syrup which T.L.C. showed consisted of starting material and product.

The syrup was taken into benzene and chromatographed on neutral alumina. Elution with benzene gave the product as a pale brown syrup which crystallised on standing to give white crystals. Recrystallisation from methylene chloride/petrol gave 11α-hydroxyprogesterone 11α-tosylate (1.91g, 70%) M.P. 153-5° (lit[116] M.P. 154-5°), ν_{max} 2950, 1700, 1670cm^{-1} N.M.R. absorptions at τ 2.12, 2.26, 2.57, 2.70, 4.26, 7.57, 8.13, 8.66, 9.36.

Δ9(11)-Progesterone.[81,82,83]

Two methods were used to detosylate the above compound.

1. 11α-Hydroxy progesterone 11α-tosylate (1g, 2.1mM) was refluxed in collidine (30ml) for one hour[81]. After cooling to room temperature the solution was poured into ether (100ml). The ether solution was washed exhaustively with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water. The solution was dried and the solvent evaporated. The residue was chromatographed on ethyl acetate-washed alumina. Elution with benzene gave a yellowish syrup which crystallised on standing to give white needles. Recrystallisation from acetone/hexane gave Δ9(11)-progesterone (0.45g, 66%) M.P. 122-3° (lit[121] M.P. 122-4°) ν_{max} 2930, 1700, 1660, 1617, 1260cm^{-1}, N.M.R. absorptions at τ 4.24, 4.45, 7.87, 8.69, 9.39.

2. 11α-Hydroxy progesterone 11α-tosylate (1g, 2.1mM) was refluxed for one hour under nitrogen in dimethyl formamide (DMF 85ml) after the addition of lithium carbonate (2.5g) and lithium bromide (3.0g)[82]. The solution was cooled to room temperature and poured into ether (300ml). The ether solution was washed exhaustively with water, dried, and the solvent evaporated to give the product. Recrystallisation from acetone/hexane gave white needles of Δ9(11)-progesterone (0.42g, 62%) M.P. 128-30°. I.R. and N.M.R. spectra confirmed the compound was identical to that prepared previously.

Stigmastadienone (Stigmasta-4,22,dien-3-one).[84,85,64,117]

Stigmasterol (1g, 2.4mM), aluminium isopropoxide (1.2g, 5.9mM) and cyclohexanone (10ml) were added to toluene (30ml) and refluxed for two hours[84,85]. The solution was cooled to room temperature, poured into water, and washed with concentrated hydrochloric acid, and water until neutral. The solution was dried and the toluene evaporated to give a yellow syrup which consisted of the product, contaminated with cyclohexanone

condensation products[84,85]. Vacuum distillation removed most of this impurity and the last residues were removedby heating on a water-bath at 80°, while passing a current of nitrogen for one hour. The product was recrystallised from ethanol to give white crystals of stigmastadienone, (0.7g, 70%) M.P.122-4°,(lit[117] M.P.124-5), νmax 3000,1660,1620cm^{-1}, N.M.R. absorptions at τ 4.27, 4.90, 8.81, 9.27.

This oxidation was repeated using Jones Reagent[64] as oxidant. Stigmasterol (1g, 2.4mM) was dissolved in acetone (20mls) and chromium trioxide in sulphuric acid (1ml of a solution containing 27g chromium trioxide in 100mls of 4M sulphuric acid) was added. The solution was stirred at room temperature for fifteen minutes, and poured into ethyl acetate (100ml). Methanol (10ml) was added to destroy excess oxidising agent. The ethyl acetate layer was washed twice with dilute hydrochloric acid, twice with saturated sodium bicarbonate solution, and water, and dried. Evaporation of the solvent gave a brown syrup, which was shown by T.L.C. to consist of the required product, contaminated with degradation products. N.M.R. spectra showed that only 40% of the product was the title compound, and no attempt was made to isolate the compound.

4,4,14α-Trimethylcholest-8-en-3β-ol (Dihydrolanosterol).[88]

Lanosterol (5g, 11.7mM) was dissolvedin ethanol (600ml) with warming, and was hydrogenated at atmospheric pressure using pre-reduced Adams catalyst (PtO_2, 0.5g) as catalyst. The reaction was stirred until there was no further uptake of hydrogen, (250mls) in 120 minutes; calculated volume is 253mls). Theplatinum was filtered off on Celite, and a white paste was obtained on evaporation of the solvent. Recrystallisation from ethyl acetate/methanol gave 24,25-dihydrolanosterol (4.5g, 88%) M.P. 144-5° (lit[88] M.P. 145°), ν max 3550, 2930cm^{-1}, N.M.R. absorptions at τ 6.6-6.9, 8.01, 8.02, 8.11, 8.13, 8.17, 9.00, 9.13.

Androst-2-en-17β-yl Acetate.[89,90]

Androstane-3β,17β-diol 17β-acetate.[118]

Androstane-17β-ol-3-one 17β-acetate (1g, 3mM) was dissolved in ethanol (70ml) and sodium borohydride (1g, 26mM) was added cautiously. The solution was stirred at room temperature for one hour. Acetic acid was added to destroy the excess sodium borohydride, and the solution volume was reduced to 25ml. Ether (100ml) was added and the ether solution was washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water. The solution was dried, and solvent evaporated to give pale yellow crystals, which were recrystallised from aqueous methanol to give androstane-3β,17β-diol 17β-acetate (0.8g, 80%) M.P. 149-51° (lit[118] M.P. 150-1°) ν max 3600, 1718, 1265, 1045cm^{-1}, N.M.R. absorptions at τ 5.2-5.6, 6.2-6.7, 7.98, 8.82, 9.20.

Androstane-3β,17β-diol 17β-Acetate 3β-Tosylate.

Androstane-3β,17β-diol 17β-acetate (0.8g, 2.4mM) was dissolved in pyridine (10ml) and p-toluene sulphonyl chloride (0.8g, 3.9mM) was added. The solution was left at room temperature for three days. The solution was poured into ice-water and the steroid extracted into ether. The ethereal solution was washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water, and dried. Evaporation of the solvent gave the product as an oil. The N.M.R. spectrum showed the product was contaminated with free acid and unreacted alcohol.

No attempt was made to purify the product, which had the following spectral figures, ν max 2930, 1710, 1600, 1360, 1260cm^{-1}, N.M.R. absorptions at τ 2.20, 2.28, 2.65, 2.73, 5.3-5.7, 7.59, 8.01, 9.22, 9.24.

The crude product was taken on to the next stage.

Androst-2-en-17β-ol 17β-acetate.[89]

The crude product (1g) from the previous reaction was dissolved in

benzene (5ml) and adsorbed on alumina and allowed to stand overnight at room temperature. Elution with benzene gave the product which was recrystallised from ether to give androst-2-en-17β-ol 17β-acetate (0.4g, approx 60%) M.P. 97-9° (lit [89] M.P. 98-9°), ν max 2900, 1713, 1260cm^{-1}, N.M.R. absorptions at τ 4.42, 5.34, 5.42, 5.50, 7.99, 9.21, 9.24. Elution with 50% chloroform/benzene gave androstane-3β,17β-diol 17β-acetate and another compound as one fraction.

Preparative T.L.C. of this fraction separated the two compounds, and I.R. and N.M.R. figuresidentified the two components as:

1. Androst-2-en-17β-ol (0.1g) M.P. 168-70°, (lit[119] M.P. 163°), N.M.R. absorptions at τ 4.41, 6.30, 6.38, 6.46, 9.26, 9.28.

2. Androstane-3β,17β-diol.17β-acetate (0.1g) M.P. 149-51° (lit[118] M.P. 150-1°), ν max 2920, 1713, 1380cm^{-1}, N.M.R. absorptions at τ 5.34, 5.42, 5.50, 5.98, 7.99, 9.24 (C-18 and C-19 methyl signals combined).

Cholesteryl Acetate.[91]

Cholesterol (1g, 2.6mM) was dissolved in pyridine (10ml) and acetic anhydride (1ml, 10mM) was added. The solution was left overnight at room temperature. The usual work-up gave the product as white crystals. Recrystallisation from aqueous methanol gave cholesteryl acetate (1.0g, 90%) M.P. 113-4° (lit[91] M.P. 114-5°), ν max 2950, 1750, 1260cm^{-1}, N.M.R. absorptions at τ 4.70, 4.76, 5.1-5.7 (complex multiplet; proton adjacent to acetate), 8.00, 9.00, 9.10, 9.20, 9.32.

Cholestanyl Acetate.[91]

Cholestan-3β-ol (1g, 2.6mM) was dissolved in pyridine (10ml) and acetic anhydride (1ml, 10mM) was added. The solution was left overnight at room temperature. The usual work-up gave the product as white crystals. Recrystallisation from acetone gave cholestanyl acetate, (1g, 90%) M.P. 109-110° (lit[91] M.P. 109-11°) ν max 2950, 1715, 1260cm^{-1}, N.M.R. absorptions at τ 5.1-5.5 (complex multiplet,) 8.00, 8.80, 9.10, 9.20, 9.35.

Allocholesteryl Acetate.[91]

Allocholesterol (1.4g, 3.6mM) was dissolvedin pyridine (20ml) and acetic anhydride (2ml, 20mM) was added. The solution was left overnight at room temperature. The usual work-up gave the product as an oil which crystallised on cooling. Recrystallisation from aqueous methanol gave allocholesteryl acetate (1.4g, 90%) M.P. 83-4.5° (lit[91] M.P. 83-4.5°) ν max 2950, 1715, 1255, 1030cm^{-1}, N.M.R. absorptions at τ 4.75 (olefinic proton and proton adjacent to acetate), 7.96, 8.94, 9.09, 9.18, 9.32.

Dehydroepiandrosterone (D.H.A.) Acetate.[92]

Dehydroepiandrosterone (5g, 17.3mM) was dissolved in pyridine (50ml) and acetic anhydride (5ml, 50mM) was added. The solution was left overnight at room temperature. The usual work-up gave white crystals. Recrystallisation from benzene gave D.H.A. acetate (4.5g, 80%), M.P. 169-71° (lit[92] M.P. 172-3°), ν max 2930, 1720, 1260cm^{-1}, N.M.R. absorptions at τ 4.5-4.7, 5.1-5.6, 7.96, 8.94, 9.11.

Androstanolone Acetate.[93]

Androstanolone (1g, 3.6mM) was dissolved in pyridine (10ml) and acetic anhydride (2ml, 20mM) was added. The solution was left overnight at room temperature. The usual work-up gave very pale yellow crystals. Recrystallisation from ethyl acetate gave androstanolone acetate (0.91g, 80%), M.P. 156-8° (lit[93] M.P. 158-9°), ν max 2950, 1703 (with shoulder at 1717) cm^{-1}, N.M.R. absorptions at τ 5.3-5.6 (complex multiplet) 7.99, 8.98, 9.20.

Testosterone Acetate.[94]

Testosterone (7g, 24.3mM) was dissolved in pyridine (50ml) and acetic anhydride (7ml, 70mM) was added. The solution was left overnight at room temperature. The usual work-up gave white crystals. Recrystallisation from aqueous acetone gave testosterone acetate (7g, 88%) M.P. 141-2.5, (lit[94] M.P. 141-25°), ν max 2930, 1715, 1660, 1265cm^{-1}, N.M.R. absorptions

at τ 4.30, 5.27, 5.38, 5.51, 7.97, 8.80, 9.17.

Dihydrolanosteryl 3-acetate.[95]

Dihydrolanosterol (1g 2.3mM) was dissolved in pyridine (10ml) and acetic anhydride (1ml, 10mM) was added. The solution was left overnight at room temperature. The usual work-up gave white crystals which were recrystallisedfrom acetone to give dihydrolanosteryl 3-acetate (1.0g, 90%) M.P. 119-20° (lit[95] M.P. 119-20°) ν max 2930, 1720, cm^{-1}, N.M.R. absorptions at τ 5.4, 5.5, 5.6, 7.96, 9.0, 9.12, 9.31.

Non-steroidal Aromatic Compounds.

Phenylcyclohexene.

1-Phenylcyclohexan-1-ol (10g, 57mM) was dissolved in benzene (50ml) and 50% sulphuric acid (10ml) was added. The two layer system was refluxed for three hours under a Dean and Starktrap. The solution was cooled and benzene (50ml) was added. The benzene solution was washed with saturated sodium bicarbonate solution, and water and dried. The solvent was evaporated, and the product was distilledunder reduced pressure to give a colourless liquid, -1phenylcyclohex-1-ene (6g, 60%), B.Pt 134-6°/ 21mm, ν max 2930, 1500, cm^{-1}, N.M.R. absorptions at τ 2.5-2.9 (maximum peak height at τ 2.74), 3.8-4.0, 7.4-7.8, 8.1-8.4. All peaks were complex multiplets.

1-Methyltetralin.[96,97,98]

1-Methyl 1-Hydroxy Tetralin.[96]

Methylmagnesium iodide was prepared by the addition of methyl iodide (1g, 7mM), in ether (5ml) tofresh magnesium turnings (0.90g, 37mM) under ether (20ml) under a nitrogen atmosphere. The flaskwas warmed gently until the reaction commenced, shown by cloudiness and spontaneous refluxing. The rest of the methyl iodide (4.68g, 33mM giving a total of 40mM) was run in, in ether (15ml), at such a rate as to keep the reaction refluxing gently. The reaction was stirred for thirty minutes after the completion of the addition of the methyl iodide. α-Tetralone (4.96g, 34mM) was dissolved in ether (20ml) and run in over a period of one hour. The solution was stirred at room temperature for a furtherhour. The solution was poured into excess ice/hydrochloric acid. The product was extracted into ether (100mls) and washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water, and dried. Evaporation of the solvent gave the product which was recrystallised from benzene to give

white rectangular prisms of 1-methyl-1-hydroxytetralin (3.30g, 60%) M.P. 88-9° (lit[96] M.P. 88-9°) ν max 3500, 2930cm^{-1}, N.M.R. absorptions at τ 2.4-2.6, 2.7-3.0, 7.2, 7.26, 7.97, 8.10, 8.16, 8.52. (methyl).

Dehydration of Above Product.[97,98]

1-Methyl 1-hydroxy tetralin (2g, 12.3mM) was added to 90% formic acid (20mls) and the cloudy mixture was heated on a boiling water bath until the solution went clear (1-2 minutes) and two separate layers formed (5 minutes). The solution was then heatedfor a further two minutes, cooled, poured into ether (100ml) and washed with saturated sodium bicarbonate solution and water. The solution was dried and the solvent evaporated to give a pale yellow oil. The product was distilled under reduced pressure to give one fraction, B.Pt 109°/16mm (lit[98] 91-5°/0.8mm), ν max 2950cm^{-1}, N.M.R. absorptions at τ 2.6-3.0, 3.2-3.6, 4.0-4.4, 7.0-7.5, 8.52. The N.M.R. showed the product consisted of two compounds, 3,4-dihydro-1-methyl naphthalein (88%) (lit[96] B.Pt 107/14mm), and 1-methylene tetralin, (12%) (lit[96] B.Pt 103°/14mm). Comparison of the integral of the olefinic peaks enabled this assignment; The major product had olefinic peak at τ 4.0-4.4, and the minor product at τ 3.2-3.6. No attempt was made to separate the two compounds.

Hydrogenation to 1-Methyl Tetralin.[98]

The mixture of products from the previous reaction was dissolved in amyl alcohol (15ml) and sodium (1.3g) was added as small pieces. The reaction mixture was heated to 150°C until all the sodium had dissolved. Upon initial addition of sodiu m there was a vigorous reaction and much effervescence. The sodium melted and could be seen on the surface as molten balls of sodium metal. After dissolution of all the sodium, the solution was cooled, and poured into petrol (B.P. 40-60°, 100ml). The solution was washed with dilute hydrochloric acid, water and dried. The solvent was evaporated to give a golden-brown oil. Distillation at

reduced pressure gave 1-methyltetralin (1.5g, 78% over the two stages), B.Pt, 94°/2mm, (lit[98] 87-8°/0.7mm), ν max 2950cm^{-1}, N.M.R. absorptions at τ 2.7-3.1 (Maximum peak height at 2.78), 7.0-9.2 (hump as with steroids), 8.74, 8.81 (methyl peaksplit by tertiary proton also attached to C-1, J 6.7H$_z$).

Alternative Method.

The reaction was repeated exactly as above until the isolation of the 1-methyl-1-hydroxy tetralin. At this stage, in an attempt to remove any α-tetralone remaining, the flask was immersedin a boiling water bath and nitrogen was drawn through the reaction product for thirty minutes. When the product was poured into formic acid for the dehydration step, two immiscible layers formed immediately showing that the nitrogen flow had caused the dehydration step to occur. The same work-up as before gave a very dark oil. This was dissolved in petrol and passed through a short alumina column to give a very pale yellow liquid, which was shown by N.M.R. to consist almost 100% of 3,4-dihydro-1-methyl naphthalene, N.M.R. absorptions at τ 4.1-4.3, 8.52.

Reduction with Pd/C.

The product (3.5g, 24.3mM) from the previous reaction was dissolved in ethyl acetate and hydrogenated at atmospheric pressure and room temperature using pre-reduced 10% Pd/C as catalyst. The reaction stopped when 550 mls of hydrogen had been taken up in 3.5 hours (calculated volume 544ml). The Pd/C was filtered off using Celite, to give a colourless solution. The solvent was evaporated to give the product as a colourless oil. The product was vacuum distilled and the fraction collected at 90°/1mm was kept as 1-methyltetralin, (3g, 84% for last stage), N.M.R. absorptions at τ 2.6-2.9, 8.74, 8.81. (methyl split by adjacent hydrogen J 6.7H$_z$).

1-Phenylcyclohexane-cis-1,2-diol.[99]

Osmium tetroxide (0.5g, 1.97mM) was dissolved in ether (10ml) and phenylcyclohex-1-ene (0.311g, 1.97mM) in ether (10ml) was added. The ether solution was stirred overnight at room temperature, during which time the solution turned black and a deposit of osmate ester collected at the flask bottom. Hydrogen sulphide was passed in to destroy the osmate ester, and the solution was stirred overnight under atmosphere of hydrogen sulphide. The ether was evaporated, and the residue dissolved in benzene (50ml) and the solution was filtered through Celite to remove osmium compounds. A clear solution resulted. The solution waswashed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water, and dried. The solvent was evaporated to give a crystalline product. The product was recrystallised from ether/hexane to give very pale yellow crystals of 1-phenylcyclohexane-cis-1,2-diol, (0.20g, 53%) M.P. 93-94.5° (lit[99] M.P. 93.5-94.5°), ν max 3600, 3550, 2930, 1500cm^{-1}, N.M.R. absorptions at τ 2.5-2.9, 7.4-7.8, 8.1-8.4. All peaks were complex multiplets.

Peracetic Acid.[108]

30% hydrogen peroxide (27g) was added cautiously to a stirred solution of acetic acid (10g) and concentrated sulphuric acid (0.1g) with the temperature maintained at 20°C. The solution was stirred overnight.

The solution is reported[108] to contain 2-2.5% of peracetic acid and this concentration was used in calculations. The solution was not standardised.

Ceric Hydroxide.

CAN (1g) was dissolved in water (5ml) and 20% sodium hydroxide solution (5ml) was added. A cloudy yellow precipitate was formed. The precipitate was filtered off, washed well with water, and dried overnight in a dessicator over calcium chloride. The pale yellow solid was

standardised by dissolution in sulphuric acid and titration with ferrous ammonium sulphate, with ferroin as indicator[19]. The standardisation showed the product contained 97% of the theoretical amount of ceric.

CAN OXIDATIONS.

Oestrone Acetate.

1. Oestrone acetate (1g, 3.2mM) was dissolved in acetic acid (18ml). CAN (7.02g, 12.8mM) was dissolved in water (2ml) and added to the acetic acid solution. The solution became dark red in colour. The colour faded rapidly, and after twenty minutes had faded to pale yellow. The solution was poured into ether (100ml) and washed with saturated sodium bicarbonate solution and water. The solution was dried and the solvent evaporated to give a pale yellow syrup, which crystallised on standing. Recrystallisation from methanol (or acetone) gave the product; 3,9α,11β-tri-hydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.9g, 70%) M.P. 190-2° (Found C61.87%, H5.91%, N3.67%, $C_{20}H_{23}NO_7$ requires C61.69%, H5.91% N3.60%) ν max 3550, 2920, 1730, 1635, 1285, 1220, 865cm^{-1}, N.M.R. absorptions at τ 2.65, 2.81, 3.10, 4.21 (11α-proton) 7.74, 8.99. Mass spectral figures m/e = 389 (P. small), 343 (P-46, $-NO_2$), 325, (P-64, $-HNO_3-H$), 283 (P-106, $CH_3CO,-HNO_3$), 257 (p-132,$-CH_3CO$,$-HNO_3$,-CHCH).

2. Oestrone acetate (1g, 3.2mM) was dissolved in acetic acid (18ml) CAN (14.04g, 25.6mM) was dissolved in water (3ml) and added to the acetic acid solution. The solution became darkred in colour. After fifteen minutes the solution (still dark red in colour) was poured into ether (100ml) and worked up as with the previous reaction to give a pale yellow syrup, which crystallised on standing. Recrystallisation from methanol gave 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (1.17g, 90%) M.P 190-2°, confirmed by I.R. and N.M.R. spectra to be identical to the compound prepared above.

T.L.C. and N.M.R. of the crude product showed that no starting material remained but a small amount of degradation had occurred.

When the reaction was repeated, with all quantities the same as above,

but for thirty minutes, extensive degradation was found to occur. (It would appear that the best time for this concentration would be fifteen minutes. The disadvantage of the small amount of degradation is more than outweighed by the increased yield of product.)

3. Oestrone acetate (1g, 3.2mM) was dissolved in acetic acid (18ml). CAN (10.53, 19.2mM) was dissolved in water(3ml) and added to the acetic acid solution. The solution became dark red in colour. After fifteen minutes, the solution, now pale yellow, was poured into ether (100ml) and worked up as usual. The product was obtained as a pale yellow syrup, which crystallised on standing. Recrystallisation from methanolgave 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (1.22g, 94%) M.P. 190-2°, confirmed by I.R. and N.M.R. spectra to be identical to the compound prepared above. T.L.C. of the crude product showed that the product consisted only of product. The N.M.R. spectrum of the crude product confirmed that no starting material remained, and no degradation had occurred.

This reaction showed the best yield of product, with least degradation was obtained using a CAN:steroid molar ratio of 6:1.

4. Oestrone acetate (1g, 3.2mM) was dissolved in propionic acid (18ml). CAN (7.02g, 12.8mM) was dissolved in water (3ml) and added to the propionic acid solution. The solution became dark red in colour. After thirty minutes the solution, changed to pale yellow in colour, was worked up in the usual manner to give a pale yellow syrup. Preparative T.L.C. enabled the isolation of the product, 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.50g, 40%) M.P. 190-2°, confirmed by I.R. and N.M.R. spectra.

5. Oestrone acetate (0.1g, 0.32mM) was dissolved in formic acid (2ml). CAN (0.7g, 1.28mM) was dissolved in water (0.2ml) and added to the formic acid. The solution was stirred at room temperature for thirty minutes.

The usual work-up gave a pale yellow syrup, which was shown by N.M.R. to contain 25% of 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate, which however was not isolated.

9β-Oestrone Acetate.

9β-Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (2ml). CAN (0.7g, 1.28mM) was dissolved in water (0.2ml) and added to the acetic acid solution. After twenty minutes, the reaction was worked up in the usual manner. The product would not crystallise. Preparative T.L.C. enabled the isolation of the product. Recrystallisation from methanol gave the same product as the previous reaction, 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.06g, 48%) M.P. 190-2° I.R., N.M.R. and T.L.C. confirmed the products were identical.

3-Hydroxyoestra-1,3,5(10)9(11)-tetraen-17-one 3-acetate.

The steroid (1g, 3.2mM) was dissolved in acetic acid (9ml). CAN (3.5g, 6.4mM) was dissolved in water (1ml) and added to the acetic acid solution. The colour faded very quickly and after five minutes the reaction was worked up in the usual manner. The product was isolated by preparative T.L.C.. Recrystallisation from methanol gave the product, 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.75g, 60%), M.P. 190-2° I.R., N.M.R. and T.L.C. confirmed the compound was the same as in the preceding oxidation reactions.

3,9α-Dihydroxyoestra-1,3,5(10)-trien-17-one 3-Acetate.

CAN (0.333g, 0.61mM) was dissolved in water (1ml) and acetic acid (3ml) was added. 3,9α-Dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (0.100g, 0.3mM) was added as solid and the solution was stirred at room temperature until colourless (30 minutes). The usual work-up gave the product as a pale yellow syrup, which was shown by TLC to consist of three compounds. Preparative T.L.C. yielded, as crystalline products:

1. 3-Hydroxyoestra-1,3,5(10)9(11)-tetraen-17-one 3-acetate, (30mgs, 30%) M.P. 125-7° (lit[57] M.P. 128-9°) confirmed by N.M.R. spectra with absorptions at τ 3.79, 7.77, 9.11.

2. 3,9α,11β-tri-hydroxyoestra-1,3,5(10)-trien-17-one 3-acetate, 11β-nitrate, (35 mgs, 30%) M.P. 190-2°, confirmed by I.R. spectrum, ν max 3550, 2920, 1730, 1635, 1285, 1220cm^{-1}, and N.M.R. absorptions at τ 4.21, 7.74, 8.99.

3. 3, 9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (25mgs, 25%), MP 166-8° (lit[73] M.P. 167-8°) confirmed by I.R. spectrum, ν max 3600, 1735, 1720cm^{-1}, and T.L.C. comparison with the starting material.

Oestradiol Diacetate.

Oestradiol diacetate (1g, 2.8mM) was dissolved in acetic acid (18ml). CAN (6.16g, 11.2mM) was dissolved in water (2ml) and added to the acetic acid solution. After 30 minutes, the solution was workedup in the usual manner to give a pale yellow froth. Preparative T.L.C. of this froth enabled the isolation of two products; 3,9α,11β,17β-tetrahydroxy-oestra-1,3,5(10)-trien 3,17β-diacetate 11β-nitrate in 50% yield , and the 9βisomer in 15% yield.

The major product was recrystallised from methanol to give 3,9α,11β,17β-tetrahydroxyoestra-1,3,5(10)-triene3,17β-diacetate 11β-nitrate (0.6g, 50%) M.P. 177-8° (Found C60.68%, H6.35%, N3.16%, $C_{22}H_{27}NO_8$ requires C60.97%, H6.24%, N3.23%), ν max 3620, 1750, 1730, 1635, 1370, 1270, 1205cm^{-1}, N.M.R. absorptions at τ 2.62, 2.78, 3.10, 4.26, 7.78, 8.00, 9.06.

The minor product would not crystallise. A colourless froth of 3, 9β,11β,17β-tetrahydroxyoestra-1,3,5(10)-triene3,17β-diacetate 11β-nitrate was obtained, ν max 3620, 1750, 1635, 1250, 1205cm^{-1}, N.M.R. absorptions at τ 2.61, 2.77, 3.11, 3.93 (C-11α-proton), 7.78, 8.00, 8.98, (C-18 Methyl).

(This compound was characterised by reduction to the corresponding

tetrahydroxy compound, see P 149-50).

Oestrone Propionate.

The steroid (0.11g 0.37mM) was dissolved in acetic acid (2ml). CAN (0.776g, 1.48mM) was dissolved in water and added to the acetic acid solution. The solution was left at room temperature for fifteen minutes, by which time the colour had faded to pale yellow. The reaction was worked up in the usual manner to give a pale yellow syrup. Preparative T.L.C. gave a pale yellow syrup which was recrystallised from methanol to give 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-propionate 11β-nitrate(53mgs, 40%), M.P. 187-8° (decomposition), (Found C62.37% H6.30% N3.58%, C_2,H_{25},NO_7 requires C62.53%, H6.20%, N3.47%), ν max 3650, 3580, 3400, 2900, 1730, 1635, 1285cm^{-1}, N.M.R. absorptions at τ 2.65, 2.80, 3.10, 4.19, 7.39, 7.51, 7.63, 7.75, 8.64, 8.76, 8.88, 9.00. N.M.R. indicated the presence of a small quantity (less than 5%) of the 9βisomer (C-18 peak at 8.89) which could not be isolated.

Oestrone Benzoate.

Oestrone benzoate (0.2g, 0.5mM) was added to acetic acid (5ml). The compound was only sparingly soluble and a suspension of the steroid in acetic acid was obtained. CAN (1.17g, 2.1mM) was dissolved in water (1ml) and added to the acetic acid. The solution was stirred for 24 hours at room temperature (to enable solution and reaction to take place). The usual work-up gave the product as a colourless froth. Preparative T.L.C. gave the product which was recrystallised from ether to give 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-benzoate 11β-nitrate (72mgs, 30%) M.P. 181-2° (Found C66.34%, H5.60%, N3.15%, $C_{25}H_{25}NO_7$ requires C66.52%, H5.54%, N3.10%), ν max 3550, 2950, 1720, 1635, 1270, 1230cm^{-1}, N.M.R. absorptions at τ 1.79, 2.10, 2.43, 2.53, 2.95, 4.14, 8.99. N.M.R. again indicated the presence of the 9βisomer in less than 5% yield.

Oestrone Methylether (3-Methoxy Oestrone).

The steroid (0.2g, 0.70mM) was dissolved in acetic acid (5ml). CAN (1.49g, 2.8mM) was dissolved in water (1ml) and was added to the acetic acid solution. The solution became deep red in colour and very quickly faded to pale yellow (less than two minutes). The usual work-up gave a pale yellow syrup. Evaporation of the solvent was done at a temperature below 40°. (It was found higher temperature resulted in extensive decomposition of the product.) Preparative T.L.C. gave the product which was recrystallised from aqueous methanol to give 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-methylether 11β-nitrate, (51mgs, 20%) M.P. 168-9° (decomposition) (Found C63.01%, H6.47%, N3.79%, $C_{19}H_{23}NO_6$ requires C63.15% H6.37% N3.88%), ν max 3580, 2930, 1635, 1280cm^{-1}, N.M.R. absorptions at τ 2.70, 2.85, 3.29, 4.17, 6.23, 8.99.

3-Meth oxyoestra-1,3,5(10),9(11)-tetraen-17-one.

The steroid (0.2g, 0.70mM) was dissolved in acetic acid (5ml). CAN (0.75g, 1.4mM) was dissolved in water (1ml) and was added to the acetic acid solution. The solution became deep red and within a few seconds faded to pale yellow. The usual work-up gave a pale yellow syrup. Evaporation of the solvent was done at a temperature below 40°.

Preparative T.L.C. gave the product which was recrystallised from aqueous methanol to give 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-methyl ether 11β-nitrate (42mgs, 15%), M.P. 168-9° (decomposition) confirmed by I.R. and N.M.R. spectra.

T.L.C. of the crude reaction product indicated extensive degradation had occurred. No attempt was made to identify the degradation products.

Oestrone Benzyl Ether (3-Benzyloxyoestrone).

The steroid (0.1g, 0.28mM) was dissolved in acetic acid (4ml). CAN (0.60g, 1.12mM) was dissolved in water (1ml) and added to the acetic acid solution. The solution became deep red and the colour faded very quickly

(two minutes). The usual work-up gave a dark-brown oil. T.L.C. indicated extensive degradation had occurred. N.M.R. indicated approximately 5% of the expected product had been formed, τ 4.22, 9.00, but the product could not be isolated from the crude product mixture.

Preparative T.L.C. enabled the isolation of 3-benzyloxyoestrone (25mgs, 25%) M.P. 128-30° (lit[60] M.P. 129-30°), confirmed by I.R. and N.M.R. spectra.

The reaction was repeated keeping all stages of the work-up at room temperature. Degradation again occurred. Starting material (25mgs, 25%) was again recovered.

Oestrone o-Nitrobenzoate.

The steroid (0.2g, 0.48mM) was added to acetic acid (60ml). The compound was only sparingly soluble and a suspension of the steroid in acetic acid was obtained. CAN (1.05g, 1.92mM) was dissolved in water (6ml), and added to the acetic acid solution. The solution was left at room temperature for forty hours, by which time no ceric remained in the solution. (This was shown by titration of 2ml aliquots of the mixture with previously standardised ferrous ammonium sulphate, with ferroin as indicator[19].) The usual work-up yielded the product. Preparative T.L.C. enabled the isolation of the product, which was recrystallised from ether to give pale yellow crystals of 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-o-nitrobenzoate 11β-nitrate (60mgs, 25%) M.P. 150-3° (Found C60.62%, H4.80%, N5.60%, $C_{25}H_{24}N_2O_9$ requires C60.48%, H4.84%, N5.65%), ν max 3600, 2930, 1730, 1640, 1540, 1500, 1280, 1260cm^{-1}, N.M.R. absorptions at τ 1.9-2.4, 2.57, 2.73, 2.93, 3.02, 4.14, 8.99.

Oestrone Tosylate.

The steroid (0.1g, 0.23mM) was dissolved in acetic acid (7ml). CAN (0.7g, 0.92mM) was dissolved in water and added to the acetic acid

solution. The solution was stirred for eight hours. The usual work-up gave the product. T.L.C. indicated only about 25% reaction had occurred. Preparative T.L.C. gave the product which was recrystallised from cyclohexane/methylene chloride to give 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-tosylate 11β-nitrate. (30mgs, 27%) M.P. 193-4° (decomposition). (Found C59.51., H5.72% N2.68%, $C_{25}H_{29}NSO_8$ requires C5964%, H5.77%, N2.78%), ν max 2930, 1725, 1635, 1600, 1385, 1270, 1220cm^{-1} N.M.R. absorptions at τ 2.21, 2.34, 2.78, 2.91, 2.63, 3.25, 3.40, 4.24, 7.58, 9.01.

3-Desoxyoestrone.

The steroid (0.05g, 0.185mM) was dissolved in acetic acid (3ml). CAN (0.55g, 0.740mM) was dissolved in water (1ml) and added to the acetic acid solution. The solution was stirred at room temperature for thirty minutes. The usual work-up yielded the product as a pale yellow syrup. Preparative T.L.C. gave the product which was recrystallised from acetone to give 9α,11β-dihydroxyoestra-1,3,5(10)-trien-17-one 11β-nitrate (31mgs, 47%) M.P. 210-14°. (Found C65.2%, H6.47%, N4.09%, $C_{18}H_{21}NO_5$ requires C65.26%, H6.34%, N4.23%), ν max 3000, 1730, 1640, 1280cm^{-1}, N.M.R. absorptions at τ 2.5-2.9 (complex multiplet). 4.16, 9.00.

N.M.R. of crude product showed the presence of a small amount (less than 5%) of the 9β isomer, C-18-methyl signal at τ 8.89; which was not isolated.

A second band on the preparative T.L.C. was examined by I.R. and N.M.R. which suggested it consisted of the following three products (total quantity 5mgs).

1. 9α-hydroxyoestra-1,3,5(10)-trien-11-17-dione, ν max 1725, 1705cm^{-1}; τ 9.12.

2. 9α-hydroxyoestra-1,3,5(10)-trien-17-one, ν max 1725cm^{-1}, τ 9.10.

3. 6β-hydroxyoestra-1,3,5(10)-trien-17-one N.M.R. absorptions at τ 6.2-6.4, 9.08.

This last compound was present in highest quantity.

The small yields prevented further investigation of this band.

2,4-Di-nitrooestrone Acetate.

The steroid (0.04g, 0.10mM) was dissolved in acetic acid (2ml). CAN (0.219g, 0.40g mM) was dissolved in water (1ml) and added to the acetic acid solution. The solution was stirred at room temperature for fifteen minutes. The usual work-up gave the product, which was shown by I.R., N.M.R. and T.L.C. to consist of starting material (0.038g, 95% return of unrecrystallised material).

The reaction was repeated with the steroid (0.1g, 0.25mM) and CAN (0.548g, 1mM) in 90% acetic acid (25ml). Titration showed no ceric remained after four days. The usual work-up gave the product which was recrystallised from aqueous methanol to give 2,4-dinitrooestrone acetate M.P. 186-8° (lit[66] M.P. 187-8.5°) confirmed by I.R. and N.M.R. spectra. T.L.C. of the crude product and of the mother liquor of the recrystallisation showed no other product was present.

2,4-Dinitrooestrone 3-Methyl Ether (2,4 Dinitro 3-methoxy Oestrone)

The steroid (0.085g, 0.22mM) was dissolved in acetic acid (9ml). CAN (0.5g, 0.88mM) was dissolved in water (1ml) and was added to the acetic acid solution. The solution was stirred at room temperature for thirty minutes. The usual work-up gave the product which was shown by I.R., N.M.R. and T.L.C. to consist only of starting material (95% return).

The reaction was repeated with the steroid (0.85g, 0.22mM) and CAN (0.5g, 0.88mM) in 90% acetic acid (25ml). Titrations showed no ceric remained after 84 hours. The usual work-up gave the product which was recrystallised from methylene chloride/hexane to give 2,4-dinitrooestrone 3-methyl ether, M.P. 119-21° (no lit M.P.) confirmed by I.R. and N.M.R.

spectra.

Only 50% return was obtained, suggesting the steroid had reacted to give a product which had been extracted during the work-up.

This was not investigated further.

17-Desoxyoestrone Acetate.

The steroid (0.05g, 0.17mM) was dissolved in acetic acid (3ml). CAN (0.36g, 0.68mM) was dissolved in water (1ml) and added to the acetic acid solution. The solution was stirred at room temperature for thirty minutes, by which time the colour had faded to very pale yellow. The usual work-up gave the product as a pale yellow syrup. Preparative T.L.C. enabled the isolation of the product. The product would not crystallise, but was shown by I.R. andN.M.R. spectra tobe 3,9α,11β-trihydroxyoestra-1,3,5(10)-triene 3-acetate 11β-nitrate (0.035g, 55%), νmax 3550, 2900, 1745, 1635, 1280cm^{-1}, N.M.R. absorptions at τ 4.29, 7.75, 9.16.

The N.M.R. spectrum again showed that about 5% of the 9β-isomer had been formed, (C-18-methyl at τ 9.08) but this product was not isolated.

3-Hydroxy-19-norcholesta-1,3,5(10)-triene 3-acetate.

The steroid (0.115g, 0.28mM) was dissolved in acetic acid (3ml). CAN (0.61g, 1.23mM) was dissolved in water (1ml) and added to the acetic acid solution. The solution was stirred at room temperature for thirty minutes. The usual work-up gave the product as a pale yellow syrup.

Preparative T.L.C. enabled the separation of the product as two components.

The major component was recrystallised from methanolto give 3,9α, 11β-trihydroxy-19-norcholesta-1,3,5(10)-triene 3-acetate 11$^{\beta}$-nitrate (24mgs, 21%) M.P. 171-2° (decomposition) (Found C69.12%, H8.41%, N2.95%, $C_{28}H_{41}NO_6$ requires C68.99%, H8.42%, N2.87%) νmax 3600, 2930, 1720, 1635cm^{-1}, N.M.R. absorptions at τ 4.27, 7.75, 9.16.

The minor component would not crystallise but was shown by I.R. and N.M.R. to be 3,9β,11β-trihydroxy-19-norcholesta-1,3,5(10)-triene 3-acetate 11β-nitrate, (11mgs, 10%) ν max 3600, 2930, 1720, 1635cm^{-1}, N.M.R. absorptions at τ 3.94, 7.75, 9.06.

3-Phenylandrost-2-en-17β-yl 17β-Acetate

3-Phenylandrost-2-en-17β-ol 17β-acetate (0.1g, 0.26mM) was dissolved in acetic acid (9mls) and CAN (0.28g, 0.52mM) in water (1ml) was added. The solution was left at room temperature for ten minutes. The orange colour of the CAN faded to pale yellow almost immediately upon addition to the steroid. The usual work-up gave a pale yellow oil. Preparative T.L.C. enabled the isolation of the product from unreacted starting material. The product was recrystallised from acetone to give 2β,3α,17β-trihydroxy 3β-phenylandrostane 17β-acetate 2β-nitrate (72mgs, 60%) M.P. 190-5° (with decomposition, softening from 180°), (Found C68.58%, H7.80%, N3.05%, $C_{27}H_{37}O_6N$ requires C68.79%, H7.86%, N2.97%) ν max 2900, 1710, 1635, 1255cm^{-1} N.M.R. absorptions at τ 2.4-2.8, 3.7-3.8, (proton on C-2 adjacent to nitrate) 5.3-5.5 (C-17-proton), 7.97, 9.19 (C-18 methyl), 9.11 (C-19 methyl). It is interesting to note the differences in the C-18 and C-19 methyl peaks. Prior to oxidation, the peaks occur at 9.25, and 9.22 respectively. Upon oxidation, the peaks occurat 9.19 and 9.11respectively. Incorporation of nitrate results in the lowering of the C-19 methyl peak by 0.11τ. This, it was found from the oestrone derivatives, appears to be the standard shift for insertion of nitrate into a position with 1,3 relationship to the angular methyl group. Examination of the structure of the starting compound and product, shows that the C-19 methyl has the same orientation relative to the aromatic ring and nitrate group as does the C-18 methyl of oestrone derivatives and therefore a similar shift would be expected. The nitrate group, being further away, has a lesser effect upon the C-18 methyl of the androstane

NOTES ON OESTRONE ACETATE OXIDATION.

Effect of light on CAN Oxidation of Oestrone Acetate.

Daylight.

Oestrone acetate (0.2g, 0.64mM) was reacted with CAN (1.4g, 2.56mM) in 90% acetic acid (50ml). Titres indicated the reaction was ended after five hours. The usual work-up gave a yellow syrup from which was obtained, by preparative T.L.C., the normal product, 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.18g, 70%) M.P. 190-2° confirmed by I.R. and N.M.R. spectra.

Darkness.

Oestrone acetate (0.2g, 0.64mM) was reacted with CAN (1.4g, 2.56mM) in 90% acetic acid (50ml). During the reaction, the solution was stirred in the dark. Titres indicated the reaction was finished after twelve hours. The usual work-up gave a yellow syrup, which, by preparative T.L.C., yielded 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate, (0.13g, 50%) M.P. 190-2°, confirmed by I.R. and N.M.R. spectra.

U.V. Light.

Oestrone acetate (0.2g, 0.64mm) was reacted with CAN (1.4g, 2.56mM) in 90% acetic acid (50ml). The solution was stirred at room temperature while being irradi ated with U.V. light, of wavelength 354nm, from a Camag T.L. 900 lamp. Care was taken to exclude day-light from the reaction. Titres showed the reaction was finished after four hours (approximately the same time as in daylight). The usual work-up gave a pale yellow syrup, which, by preparative T.L.C. yielded 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.16g, 60%) M.P. 190-2° confirmed by I.R. and N.M.R. spectra.

Effect of Elevated Temperature.

Oestrone acetate (0.1g, 0.32mM) was reacted with CAN (0.7g, 1.28mM)

in 90% acetic acid (25mls), at 80°C. Titres showed the reaction was finished in five hours, approximately the same time as required at room temperature, indicating no increase in rate with temperature. The usual work-up gave the product, 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.04g, 30%) M.P. 190-2°, confirmed by I.R. and N.M.R. spectra. T.L.C. of the crude product showed much degradation of the product had occurred during the reaction.

Deoxygenated Solvents

Acetic acid (100ml) was refluxed overnight under a stream of nitrogen to deoxygenate the solvent. Water was treated similarly. Oestrone acetate (0.1g, 0.32mM) was oxidised with CAN (0.7g, 1.28mM) in the usual manner, using the above solvents to make up the reaction solvent. When 25mls of 90% acetic acid was used as solvent, the reaction again took five hours (shown by titres), showing atmospheric oxygen did not alter the reaction rate. The usual work-up yielded the usual product, 3,9α,11β-trihydroxyoestra-1,3,5(10)trien-17-one 3-acetate 11β-nitrate, M.P. 190-2° in the usual yield (0.08g, 60%), showing that, as with the rate, no change occurred on removal of atmospheric oxygen from the reaction mixture.

Repetition of the reaction, using the same deoxygenated solvents, but under an atmosphere of nitrogen, resulted in the same product, yield and rate, confirming the previous results.

Investigation of Mechanism of CAN Oxidation of Oestrone Acetate.

Proof of Radical Step.

Oestrone Acetate and CAN in the Presence of Acrylamide.

Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (9ml) and acrylamide (0.95g, 13mM) was added. CAN (0.7g, 1.28mM) was dissolved in water (1ml) and added to the acetic acid solution. The solution was stirred at room temperature until the colour had faded to very pale yellow (3 hours). The usual work-up yielded the product as brown crystals. N.M.R. and T.L.C. showed the reaction had not been totally stopped, as the product was oestrone acetate, with approximately 5% of the expected product also present. T.L.C. showed a complex reaction had occurred. In addition to the two steroid spots, much streaking causedby acrylamide and polymerised acrylamide was shown on development.

When the reaction was repeated with the same proportions and conditions but with acrylonitrile (2ml) in place of acrylamide, the normal oxidation product was obtained, in 25% yield. The reaction was again found to be slower, requiring two hours to go to completion.

3-Hydroxyoestra-1,3,5(10)9(11)-tetraen-17-one 3-Acetate with CAN in the Presence of Acrylamide.[57]

The steroid (0.1g, 0.32mM) was dissolved in acetic acid (9ml) and acrylamide (0.95g, 13mM) was added. CAN (0.35g, 0.64mM) was dissolved in water (1ml) and added to the acetic acid solution. The reaction was stirred at room temperature for one hour. The usual work-up gave a brown syrup as product.

Preparative T.L.C. yielded two crystalline compounds.

1. 3-Hydroxyoestra-1,3,5(10)9(11)-tetraen-17-one 3-acetate, (0.050g, 50%) M.P. 125-7° (lit[57] M.P. 128-9°) confirmed by I.R. and N.M.R. spectra.

2. 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate

(0.51g, 40%) M.P. 190-2°, confirmed by I.R. and N.M.R. spectra.

3,9α-Dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate and CAN in the Presence of Acrylamide.[57,73]

Acrylamide (0.95g, 13mM) was dissolved in acetic acid (9ml). CAN (0.33g, 0.6mM) was dissolved in water (1ml) and added to the acetic acid solution. The steroid (0.1g, 0.3mM) was added as solid, and the solution was stirred at room temperature for one hour. The usual work-up yielded a brown syrup as product. Preparative T.L.C. yielded three crystalline compounds;

1. 3-Hydroxyoestra-1,3,5(10)9(11)-tetraen-17-one 3-acetate (14mgs, 15%) M.P. 126-7° (lit[57] M.P. 128-9°) confirmed by I.R. and N.M.R. spectra, with absorptions at τ 3.79, 7.77, 9.11.

2. 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (51mgs, 40%) M.P. 190-2°, confirmed by I.R. and N.M.R. spectra.

3. 3,9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate, (30mgs, 30%) M.P. 166-8° (lit[73] M.P. 167-8°) confirmed to be identical with starting material by T.L.C. and N.M.R. spectrum.

Proof of Reaction at C-9 (Benzylic Carbon).

3-Hydroxy-9β-oestra-1,3,5(10)-trien-11,17-dione 3-acetate and CAN.[101]

The steroid (0.1g, 0.31mM) was dissolved in acetic acid (9ml). CAN (0.34g, 0.62mM) was dissolved in water (1ml) and added to the acetic acid solution. The solution was stirred at room temperature for thirty minutes. The usual work-up yielded a brown syrup as the product, shown by T.L.C. to consist of two compounds in addition to degradation products. Preparative T.L.C. yielded the two products.

1. 3-Hydroxy-9β-oestra-1,3,5(10)-triene-11,17-dione 3-acetate (75mgs, 75%) which would not crystallise, ν max 2920, 1740, 1695, 1250cm^{-1}, N.M.R. absorptions at τ 6.3, 7.75, 9.08.

2. 3,9α-dihydroxyoestra-1,3,5(10)-trien-11,17-dione 3-acetate (16mgs,

15%) M.P. 325-40° (with decomposition), (lit[125] M.P. 235-43°) ν max 3550, 2930, 1750-1700cm^{-1}, N.M.R. absorptions at τ 2.53, 2.68, 2.73, 3.00, 3.12, 7.73, 9.14. This product was confirmed by comparison, on T.L.C., with authentic compound.

3-Hydroxy-9α,11-epoxyoestra-1,3,5(10)-trien 17-one 3-Acetate and CAN.

CAN (0.34g, 0.62mM) was dissolved in water (1ml) and acetic acid (9ml) was added. The steroid (0.1g, 0.31mM) was added as solid in several portions. The solution was stirred at room temperature for fifteen minutes. The usual work-up yielded a pale yellow syrup as product, which was shown by T.L.C. to consist of two compounds; starting material and product.

Preparative T.L.C. yielded the product, 3-hydroxy-9β-oestra-1,3,5(10)-triene-11,17-dione 3-acetate (50mgs, 50%). The product would not crystallise, and was identified by N.M.R. spectrum, and T.L.C. comparison with the authentic compound.

3-Hydroxy-9α,11-epoxyoestra-1,3,5(10)-trien-17-one 3-acetate and Nitric Acid.

Nitric acid (0.25ml of 70% nitric acid, 3.72mM) was added to water (1ml) and acetic acid (9ml). The steroid (0.1g, 0.31mM) was added as solid in severalportions. The solution was stirred at room temperature for fifteen minutes. The usual work-up yielded a pale yellow syrup as product. T.L.C. showed this consisted of starting material and product which was isolated by preparative T.L.C., and identified as being 3 hydroxy-9β-oestra-1,3,5(10)-trien-17-one 3-acetate, which would not crystallise. The N.M.R. spectrum and T.L.C. showed the product was identical to the compound prepared above, and to the authentic compound.

Effect of activating groups,[48]

Several oestrone derivatives were reacted with CAN using lower concentrations than in the preparative reactions. This resulted in slower reactions than before and it was found possible to follow the uptake of

ceric by titration of ceric with ferrous ammonium sulphate, with ferroin as indicator.

The oestrone derivative(1mm) was reacted with the required quantity of ceric in 90% acetic acid (50ml). 2ml samples were taken every 30 minutes and titrated with previously standardised ferrous ammonium sulphate using ferroin as indicator to find the ceric concentration.

The following results were obtained.

Compound	Quantity(g)	Quantity of Ce(IV)(g)	Mole-equivalents	Time to total reaction (hours)
Oestrone acetate	0.31	2.19	4	4.5
Δ9(11)-Oestrone acetate	0.31	1.1	2	1.25
3-Methoxy oestrone	0.28	2.19	4	1
3-Desoxy oestrone	0.25	2.19	4	9
Oestrone benzoate	0.39	2.19	4	24
Oestrone o-nitrobenzoate	0.44	2.19	4	40
3-Methoxy-2,4-dinitro oestrone	0.37	2.19	4	No reaction

The results are seen to be in general agreement with those of Murti & Pati[48]. The effect of substituents on aromatic rings appears to follow the same trend as that applying in electrophilic substitution reactions ie substituted activating groups cause an increase in reaction rate, and deactivating groups cause a decrease in rate, as measured by the rate of ceric consumption.

Essential Requirements for Oxidation.

1. Aromatic Ring.
2. Benzylic Hydrogen.
3. Ceric.
4. Nitrate.
5. Ammonium.
6. Water.
7. Acid

A series of reactions was run to find how essential each of the above species is for successful oxidation of oestrone acetate.

Headings 1, and 2, concerning the aromatic ring, and the benzylic hydrogen are dealt with elsewhere in this thesis; therefore only headings 3, to 7, will be dealt with in this section.

Requirement of Ceric.

OestroneAcetate with Nitric Acid.[56]

Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (4ml). 70% nitric acid (0.5ml), equivalent in nitrate content to the required quantity of CAN (0.7g) was added and the solution was stirred at room temperature for twenty minutes. The usual work-up yielded the product as white crystals. T.L.C. of the crude product showed a small amount of hydrolysis had occurred. Recrystallisation from aqueous methanol gave oestrone acetate (0.090g, 90%) M.P. 123-4° (lit[56] M.P. 123-4°) indicating no reaction had occurred.

Oestrone Acetate with Cerous Acetate/Nitric Acid

Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (4ml). Cerous acetate (0.4g) was dissolved in 70% nitric acid (0.5ml) and the solution was diluted to 1ml with water. The two solutions were mixed and stirred at room temperature for twenty minutes. The usual work-up yielded

a white crystalline product which was shown by T.L.C. to consist predominantly of oestrone acetate with a small amount (less than 5%) of oestrone (hydrolysis of oestrone acetate). This again indicated no reaction had occurred. The product was recrystallised from aqueous methanol to yield oestrone acetate (0.090g, 90%) M.P. 123-4°.

3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-Acetate with Nitric Acid[57]

The steroid (0.1g, 0.32mM) was dissolved in acetic acid (4ml) and 70% nitric acid (0.25ml) equivalent in nitrate content to the required quantity of CAN (0.35g) was added, and the solution was stirred at room temperature for twenty minutes. The usual work-up yielded a white crystalline product, which was recrystallised from aqueous methanol to give unchanged starting material, 3-hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate (0.090g, 90%) M.P. 125-7° (lit[57] M.P. 128-9°) ν max 2930, 1750, 1730, 1210cm^{-1}, N.M.R. absorptions at τ 3.79, 7.77, 9.11.

3,9α-Dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate with Nitric Acid[57]

70% Nitric acid (0.25ml) was added to acetic acid (9ml) and the steroid (0.1g, 0.3mM) was added as solid. The solution was stirred at room temperature for fifteen minutes. The usual work-up yielded pale brown crystals as product. T.L.C. showed this consisted of starting material and one product. Preparative T.L.C. enabled the isolation as crystalline products of:

1. 3-Hydroxyoestra-1,3,5(10),9(11)-tetraen-17-one 3-acetate (50mgs, 53%) M.P. 125-7° (lit[57] M.P. 128-9°), N.M.R. absorptions at τ 3.78, 7.77, 9.10.
2. 3,9α-Dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (25mgs, 25%) M.P. 167-8°, confirmed by I.R. and N.M.R. spectra and T.L.C., to be identical to the starting material.

Requirement of Nitrate and Ammonium Ions.

Oestrone Acetate with Ceric Hydroxide.

Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (1ml). Ceric hydroxide (0.27g, 1.28mM) was added with stirring to acetic acid (4ml). A cloudy yellow suspension was obtained. Addition of water (1ml) left much of the ceric in suspension. The oestrone acetate solution was added, and the resulting cloudy yellow solution was stirred at room temperature for one hour. The usual work-up yielded a product which was shown by I.R. and N.M.R. spectra, and T.L.C. to consist of oestrone acetate, although streaking with no clear spots, of the T.L.C. plate indicated some reaction or degradation had occurred. The product was recrystallised from aqueous methanol to give oestrone acetate(0.085g, 85%). No other product could be isolated from the reaction mixture.

Oestrone Acetate with Ceric Hydroxide/Nitric Acid.

Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (4ml). Ceric hydroxide (0.27g, 1.28mM) was dissolved in 70% nitric acid (0.5ml) to give a clear yellow solution. The two solutions were mixed and stirred at room temperature until the solution was colourless (20 minutes). The usual work-up yielded as main product, after preparative T.L.C. and recrystallisation from acetone, 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (74mgs, 61%) M.P. 190-2°, ν max 3550, 2920, 1730, 1635, 1285, 1220cm^{-1}, N.M.R. absorptions at τ 4.21, 7.74, 8.99.

Oestrone Acetate with Ceric Ammonium Sulphate.[56]

Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (1ml). Ceric ammonium sulphate (0.8g, 1.28mM) was added to acetic acid (4mls) with stirring to give a yellow suspension. Increase in the volume to 20mls failed to give a clear solution. Addition of water also failed to give complete solution of the ceric.

The steroid solution and the original ceric solution, diluted by the addition of water (1ml), were mixed to give a total volume of 6mls. The resulting cloudy yellow solution was stirred at room temperature for one hour. The usual work-up yielded a white crystalline product which was shown by T.L.C. to consist only of unchanged starting material and about 5% of oestrone (hydrolysed starting material). Recrystallisation from aqueous methanol give oestrone acetate(0.085g, 85%) M.P. 123-4° (lit[56] M.P. 123-4°) confirmed by I.R. and N.M.R. spectra.

Oestrone Acetate with Ceric Ammonium Sulphate/Nitric Acid.

Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (16ml). Ceric ammonium sulphate (0.8g, 1.28mM) was dissolved in 50% nitric acid (4ml) to give a clear orange solution. The two solutions were mixed and stirred at room temperature until colourless (1 hour). The usual work-up gave as main product, after preparative T.L.C. and recrystallisation from acetone, 3,9α,11β-trihydroxyoestra-1,3,5(10)trien-17-one 3-acetate, 11βnitrate (60mgs, 50%) M.P. 190-2° confirmed by I.R. and N.M.R. spectra.

Oestrone Acetate with Ceric Sulphate.[56]

Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (1ml). Ceric sulphate solution (13mls of 0.1N$CeSO_4$ in 1N sulphuric acid, 1.3mM) was added to acetic acid (13ml) to give a solution approximately 50% in acetic acid. The solution immediately turned cloudy yellow due to the precipitation of ceric. The steroid solution was added and the cloudy yellow suspension was stirred at room temperature for one hour. The usual work-up yielded a white crystalline product. T.L.C. showed no reaction had occurred other than a small amount of hydrolysis of the acetate group. Recrystallisation from aqueous methanol gave oestrone acetate, (0.080g, 80%) M.P. 123-4° (lit[56] M.P. 123-4°) confirmed by I.R. and N.M.R. spectra.

Oestrone Acetate with Ceric Sulphate/Nitric Acid.

Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (16ml). Ceric sulphate (0.45g, 1.28mM) was dissolved in 50% nitric acid (4ml) to give a clear orange solution. The two solutions were mixed and stirred at room temperature till colourless (1 hour). The usual work-up gave a pale yellow syrup. Preparative T.L.C. and recrystallisation from acetone gave 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11βnitrate (65mgs, 55%) M.P. 190-2°, confirmed by I.R. and N.M.R. spectra.

Oestrone Acetate with Ceric Hydroxide/Hydrochloric Acid.

Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (4ml). Ceric hydroxide (0.27g, 1.28mM) was dissolved in concentrated hydrochloric acid (1ml) to give a slightly cloudy deep red solution. The two solutions were mixed and stirred at room temperature until the solution was colourless (30 minutes). The usual work-up yielded a pale brown syrup. T.L.C. indicated much reaction and degradation had occurred but the only isolable product was oestrone acetate. Preparative T.L.C. gave 50mgs, 50% of unchanged oestrone acetate, confirmed by I.R. and N.M.R. spectra.

Oestrone Acetate with Ceric Hydroxide/Hydrochloric Acid in the Presence of Ammonium Nitrate.

Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (4ml). Ceric hydroxide (0.27g, 1.28mM) was dissolved in concentrated hydrochloric acid (0.5ml) to give a deep red solution, and ammonium nitrate (0.5g) equivalent in nitrate content to the required quantity of CAN, was dissolved in water (0.5ml). The two aqueous solutions were simultaneously added to the acetic acid solution. The deep red colour immediately faded to pale yellow. The solution was stirred at room temperature for five minutes. The usual work-up yielded the product as a brown syrup which crystallised on standing. T.L.C. and N.M.R. indicated the product was predominantly oestrone acetate (70%) but 3,9α,11β-trihydroxyoestra-

1,3,5(10)-trien-17-one 3-acetate 11β-nitrate was also present in about 10% yield. (Note: addition of ammonium nitrate to ceric hydroxide in concentrated hydrochloric acid also causes the colour to fade to pale yellow. Without oestrone acetate the colour change takes about ten minutes. With oestrone acetate the change is immediate, therefore some of the colour change must be due to reaction between ceric and oestrone acetate.)

Oestrone Acetate with Ceric Hydroxide/Perchloric Acid.

Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (4ml). Ceric hydroxide (0.27g, 1.28mM) was dissolved in 70% perchloric acid (1ml) to give a cloudy yellow solution (cloudiness due to incomplete solution). The steroid and ceric solutions were mixed and stirred at room temperature until the solution was colourless (20 minutes). The usual work-up yielded a dark-brown oil. T.L.C. indicated extensive degradation had occurred and the only isolable product was oestrone acetate. Preparative T.L.C. yielded 40mgs (40%) of oestrone acetate, confirmed by I.R. and N.M.R. spectra.

Oestrone Acetate with Ceric Hydroxide/Perchloric Acid in the Presence of Ammonium Nitrate.

Oestrone acetate (0.1g, 0.32mM) was dissolved in acetic acid (5ml). Ceric hydroxide (0.27g, 1.28mM) was dissolved in 70% perchloric acid (1ml) to give a yellow solution. Ammonium nitrate (0.6g, 7.5mM) equivalent in nitrate content to the required quantity of CAN, was dissolved in water (1ml). The aqueous solutions were added to the acetic acid solution to give a yellow suspension. The solution was stirred at room temperature for thirty minutes. The usual work-up yielded the product as a pale brown syrup which crystallised on standing. T.L.C. and N.M.R. indicated the product was predominantly oestrone acetate, but a small amount (less than 5%) of 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate was also present.

The above series of reactions showed that the presence of nitrate ions was necessary for isolable products to be obtained from ceric oxidation of oestrone acetate. In the absence of nitrate ions, extensive degradation occurred and no products could be isolated or identified.

The reactions between oestrone acetate and ceric sulphate, and ceric hydroxide, with nitric acid showed that the presence of ammonium ions was not necessary for the ceric oxidation to occur.

Requirement of Water.

Acetic Acid (Anhydrous).[56]

Oestrone acetate (0.1g, 0.32mM) was dissolved in anhydrous acetic acid (5ml). CAN (0.7g, 1.28mM) was dissolved in anhydrous acetic acid (20ml). The solutions were mixed and stirred overnight at room temperature. The solution remained orange in colour. The solution was poured into ether and worked up in the usual manner to give white crystals. Recrystallisation from aqueous methanol gave oestrone acetate (0.09, 90%) M.P. 122-4° (lit[56] M.P. 123-4°) confirmed by I.R. and N.M.R. spectra.

T.L.C. of the crude product showed a very faint shadow indicating that a very small amount of the expected product, 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate, had been formed.

Methyl Ethyl Ketone.[56]

Oestrone acetate (0.05g, 0.16mM) was dissolved in methyl ethyl ketone (2ml). CAN was added to methyl ethyl ketone (2ml). The ketone turned orange indicating that solution of some of the ceric had occurred. The two solutions were mixed and stirred at room temperature for one hour. The usual work-up yielded white crystals. Recrystallisation from aqueous methanol gave oestrone acetate (40mgs 80%) M.P. 123-4° (lit[56] M.P. 123-4°) confirmed by T.L.C. and I.R. and N.M.R. spectra.

T.L.C. of the crude product gave a very faint shadow, corresponding

to the expected product. This reaction was repeated overnight using the same quantities and conditions. The same result was obtained.

Both reactions gave a very smallyield of the oxidation product due to the presence of a small amount of water in the solvents.

Requirement of Acid.

Dimethyl Acetamide.[56]

Oestrone acetate (0.05g, 0.16mM) was dissolved in dimethyl acetamide (4ml). CAN (0.35g, 0.64mM) was dissolved in water (1ml) and was added to the steroid solution. The solutions were immiscible and it was found necessary to stir vigorously to prevent separation into two phases. After stirring for one hour, the usual work-up yielded the product as a yellow syrup which gave white crystals on cooling. Recrystallisation from aqueous methanol gave oestrone acetate (0.045g, 90%) M.P. 123-4° (lit[56] M.P. 123-4°), confirmed by I.R. and N.M.R. spectra. T.L.C. of the mother liquors showed that some of the expected product had been formed. N.M.R. confirmed the presence of 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11βnitrate in approximately 2% yield.

Dimethyl formamide.[56]

Oestrone acetate (0.05g, 0.16mM) was dissolved in dimethyl formamide (2ml). CAN (0.35g, 0.64mM) was dissolved in water (1ml). The solutions were mixed and stirred at room temperature for one hour. The usual work-up gave, after recrystallisation from aqueous methanol, white crystals of oestrone acetate (0.04g, 80%) M.P. 123-4° (lit[56] M.P. 123-4°). T.L.C. and N.M.R. of the crude product and the mother liquor from the recrystallisation showed that 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate, 11β-nitrate had been formed in approximately 5% yield.

Acetone

Oestrone acetate (0.05g 0.16mM) was dissolved in acetone (2ml).

CAN (0.35g, 0.64mM) was dissolved in water (1ml). The two solutions were mixed and stirred at room temperature for one hour, during which time the solution changed colour from orange to dark-red. The usual work-up yielded a brown syrup. T.L.C. and N.M.R. indicated considerable degradation had occurred. Preparative T.L.C. yielded the normal product, 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (27mgs, 40%) M.P. 190-2°, confirmed by I.R. and N.M.R. spectra. T.L.C. showed the presence of a small quantity (less than 5%) of oestrone formed by the hydrolysis of starting material.

Methyl ethyl ketone.

Oestrone acetate (0.05g, 0.16mM) was dissolved in methyl ethyl ketone (propan-2-one, 2ml). CAN (0.35g, 0.64mM) was dissolved in water (1ml). The solutions were mixed, and were found to be only partially miscible. Vigorous stirring at room temperature enabled sufficient mixing to occur for the reaction to proceed. After 20 minutes the orange colour had faded to pale yellow. The usual work-up gave, after preparative T.L.C., 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (27mgs, 40%) M.P. 190-2°, confirmed by I.R. and N.M.R. spectra.

T.L.C. of the crude product showed extensive degradation had occurred.

Methanol.[56]

Oestrone acetate (0.05g, 0.16mM) was dissolved in methanol (2ml). CAN (0.35g, 0.64mM) was dissolved in water (1ml). The solutions were mixed, and there was an immediate precipitation of much ceric to give a cloudy yellow suspension. The suspension was stirred at room temperature for 20 minutes. The usual work-up gave white crystals, which were recrystallised from aqueous methanol to give oestrone acetate (0.045g, 90%) M.P. 123-4° (lit[56] M.P. 123-4°). N.M.R. and T.L.C. of the crude product, and the mother liquors showed that no 3,9α,11β-trihydroxy-oestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate had been formed.

T.L.C. showed the presence of a small quantity (less than 5%) of oestrone formed by the hydrolysis of starting material.

t-Butanol.[56]

Oestrone acetate(0.05g, 0.16mM) was dissolved in t-butanol (2ml). CAN (0.35g, 0.64mM) was dissolved in water (1ml). The solutions were mixed to give a slightly cloudy orange solution which was stirred at room temperature for one hour. The usual work-up yielded white crystals which were recrystallised from aqueous methanol to give oestrone acetate (40mgs 80%) M.P. 122-4° (lit[56] M.P. 123-4°) N.M.R. and T.L.C. of the crude product and the mother liquor of the recrystallisation showed that 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate had been formed in approximately 5% yield. T.L.C. showed the presence of a small amount (less than 5%) of oestrone, formed by the hydrolysis of starting material.

Dioxan.

Oestrone acetate (0.16mM) was dissolved in dioxan (2ml). CAN (0.35g, 0.64mM) was dissolved in water (1ml). The solutions were mixed and stirred at room temperature for one hour, during which time the red colour faded to orange. The usual work-up gave a pale yellow syrup. Preparative T.L.C. and recrystallisation from methanol gave 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate, 11β-nitrate (17mgs 25%), M.P. 190-2°, confirmed by I.R. and N.M.R. spectra.

Tetrahydrofuran.[56]

Oestrone acetate (0.05g, 0.16mM) was dissolved in tetrahydrofuran (2ml). CAN (0.35g, 0.64mM) was dissolved in water (1ml). The solutions were mixed and stirred at room temperature for one hour. The usual work-up gave white crystals, which were recrystallised from aqueous methanol to give oestrone acetate (0.04g, 80%) M.P. 123-4° (lit[56] M.P. 123-4°) N.M.R. and T.L.C. of the crude product and the mother liquor from the

recrystallisation showed that 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate had been formed in approximately 5% yield.

Ether.[56]

Oestrone acetate (0.05g, 0.16mM) was dissolved in ether (2ml). CAN (0.35g, 0.64mM) was dissolvedin water (1ml). The two solutions were combined and gave two immiscible layers. The combined immiscible solutions were stirred vigorously (to encourage mixing) at room temperature for one hour. The usual work-up gave white crystals which were recrystallised from aqueous methanol to give oestrone acetate (0.045g, 90%) M.P. 123-4° (lit[56] M.P. 123-4°). N.M.R. and T.L.C. of the crude product, and the mother liquor of the recrystallisation showed no 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11βnitrate had been formed.

Benzene,[56]

The above reaction was repeated, with the same quantities and conditions, with the exception that the steroid solvent was changed to benzene. T.L.C. and N.M.R. showed no 3,9α,11β-trihydroxyoestra-1,3,5(10)-triene-17-one 3-acetate 11β-nitrate had been formed, and oestrone acetate was recrystallised from aqueous methanol to give white crystals, M.P. 122-4° (lit[56] M.P. 123-4°).

Non-Aromatic Steroids.

Cholesterol.[120]

Cholesterol (1g, 2.6mM) was dissolved in acetic acid (20ml). CAN (2.80g, 5.2mM) was dissolved in water (2ml) and added to the acetic acid solution. The solution was stirred overnight at room temperature to give a colourless solution. The usual work-up yielded a pale brown syrup. T.L.C. showed much degradation had occurred. The T.L.C. plate showed one spot with streaking trailing back to the starting point. The I.R. spectrum had the characteristic nitrate peaks at 1640, 1280, 860cm^{-1}, due to decomposition products which could not be isolated.

The syrup was dissolved in benzene and adsorbed on alumina. Elution with benzene gave the product. Recrystallisation from methanol gave cholest-4-enone (0.80g, 80%) M.P. 78-81° (lit[120] M.P. 78°) ν max 2950, 1660, 1615cm^{-1}, N.M.R. absorptions at τ 4.26, 8.81, 9.08, 9.18, 9.29.

The reaction was repeated with double the quantity of ceric (5.60g, 10.4mM) and a colourless solution was again obtained. The product was again cholest-4-enone in 70% yield. This was repeated several times and it was found cholesterol could readily consume a great excess of ceric (up to 20 mole equivalents was examined) undergoing extensive degradation to give cholest-4-enone as the only isolable product.

Δ4-Cholesterol (Allocholesterol).[120]

Allocholesterol (1g 2.6mM) was dissolved in acetic acid (20ml). CAN (2.80g 5.2mM) was dissolved in water (2ml), and added to the acetic acid solution. The solution was stirred overnight at room temperature to give a colourless solution. The usual work-up gave a pale-brown syrup. T.L.C. indicated this was predominantly cholest-4-enone, with degradation products. The syrup was dissolved in benzene and chromatographed on alumina; elution with benzene gave the product, which was recrystallised

from methanol to give cholest-4-enone (0.75g, 75%) M.P. 79-81° (lit[120] M.P. 78°) confirmedby I.R. and N.M.R. spectra.

I.R. spectrum of the crude product again showed nitrate peaks, but these products were in very small yield and could not be isolated from the degradation product.

The oxidation was repeated with double the quantity of ceric (5.60g, 10.4mM) and a colourless solution was again obtained. The product was again cholest-4-enone in 70% yield.

Cholest-5-en-3-one.[120]

Cholest-5-enone (1g, 2.6mM) was dissolved in acetic acid (20ml). CAN (5.60g 10.4mM) was dissolved in water (2ml) and added to the acetic acid solution. The solution was left stirring overnight to give a colourless solution. The usual work-up gave a pale brown syrup. T.L.C. showed this was predominantly cholest-4-enone, with degradation products. The syrup was dissolved in benzene and chromatographed on alumina. Elution with benzene gave the product which was recrystallised from methanol to give cholest-4-enone (0.525g, 52.5%) M.P. 78-81° (lit[120] M.P. 78°) confirmed by I.R. and N.M.R. spectra.

The I.R. spectrum of the crude product again showed the presence of nitrate but these compounds could not be isolated from the degradation products.

Cholest-4-enone.[120]

Cholest-4-enone (1g, 2.6mM) was dissolved in acetic acid (20ml). CAN (2.80g 5.2mM) was dissolved in water (2ml) and added to the acetic acid solution. The solution was stirred overnight at room temperature to give a colourless solution. The usual work-up gave a pale-brown syrup. T.L.C. indicated this consisted of unchanged starting material with degradation products. The product was dissolved in benzene and chromatographed on alumina. Elution with benzene gave the product which was recrystallised

from methanol to give cholest-4-enone (0.50g, 50%) M.P. 78-81° (lit[120] M.P. 78°) confirmed by I.R. and N.M.R. spectra.

The reaction was repeated using the same quantities of reagents, but the reaction was stopped after only three hours. The solution was still orange in colour. The usual work-up yielded the product, cholest-4-enone in 90% yield. Again no other isolable products were obtained.

Cholesteryl Acetate.[91]

Cholesteryl acetate (1g, 2.3mM) was dissolved in acetic acid (20ml). CAN (2.55g, 4.7mM) was dissolved in water (2ml) and added to the acetic acid solution. The solution was stirred overnight at room temperature to give a pale yellow solution. The usual work-up gave a pale-brown syrup, T.L.C. of which showed extensive degradation had occurred. The syrup was recrystallised from acetone to give cholesteryl acetate (0.50g, 50%) M.P.113-4° (lit[91] M.P. 114-5°) ν max 2930, 1720cm^{-1}, N.M.R. absorptions at τ 4.60, 4.66, 5.1-5.7, 8.00, 9.00, 9.10, 9.32.

The reaction was repeated with double the quantity of CAN (5.10g, 9.3mM) with all other quantities unchanged. The usual work-up gave a brown syrup which was dissolved in benzene and chromatographed on alumina. Elution with hexane:benzene (1:1) mixture and recrystallisation from acetone gave cholesteryl acetate (0.40g, 40%) M.P. 113-4° (lit[91] M.P. 114-5°) confirmed by I.R. and N.M.R. spectra. No other product was obtained on further elution.

Δ4-Cholesteryl Acetate (Allocholesteryl Acetate).[91]

Δ4-Cholesteryl acetate (1g, 2.3mM) was dissolved in acetic acid (50ml). Some difficulty was experienced in obtaining complete solution. CAN (2.55g, 47mM) was dissolved in water (5ml), and added to the steroid solution. The solution was stirred at room temperature for four hours, by which time titration showed no ceric remained. The usual work-up gave

a yellow syrup, T.L.C. of which showed the only product was unchanged starting material. Streaking indicated some degradation had occurred. The syrup was dissolved in benzene and adsorbed on alumina. Elution with hexane, and hexane:benzene (1:1) mixture gave the product which was recrystallised from aqueous methanol to give Δ4-cholesteryl acetate (0.5g, 50%) M.P. 82-4° (lit[91] M.P. 83-45°) ν max 2950, 1715cm^{-1}, N.M.R. absorptions at τ 4.75 (olefinic and C-3 protons), 7.96, 8.94, 9.09, 9.18, 9.32.

No further products were obtained on further elution.

Cholestanyl Acetate.[91]

Cholestanyl acetate (0.1g 0.23mM) was dissolved in acetic acid (20ml). Some difficulty was experienced in obtaining solution. CAN (0.25g, 0.47mM) was dissolved in water (5ml) and added to the acetic acid solution. The solution was stirred until titres showed no ceric remained (60 hours). The usual work-up gave a brown syrup which crystallised on cooling. T.L.C. showed this consisted of starting material with cholestanol (hydrolysed starting material) and cholestanone present in very minor quantities. The product was recrystallised from ethyl acetate/methanol to give cholestanyl acetate (0.07g, 70%) M.P. 108-109° (lit[91] M.P. 109-11°) ν max 2950, 1715cm^{-1} N.M.R. absorptions at τ 5.3, 8.0, 9.10, 9.20, 9.35.

Dehydroisoandrosterone (D.H.A.) Acetate.[92]

D.H.A. acetate (1g, 3mM) was dissolved in acetic acid (30ml). CAN (3.32, 6mM) was dissolved in water (3ml) and added to the acetic acid solution. The solution was stirred at room temperature till titration showed that no ceric remained (6 hours). The usual work-up gave a brown syrup, T.L.C. of which showed extensive degradation had occurred. Preparative T.L.C. enabled the isolation of unchanged starting material, which was recrystallised from benzene to give D.H.A. acetate (0.60g, 60%) M.P. 168-80° (lit[92] M.P. 172-3°) ν max 2930, 1720, 1260cm^{-1}, N.M.R.

absorptions at τ 4.57, 4.63, 5.1-5.7, 8.00, 8.95, 9.14.

The reaction was repeated using the same reagent quantities, but with different reaction times. Reaction for fifteen hours yielded the same product in 50% yield. Reaction for ninety minutes again yielded unchanged starting material in 80% yield.

Testosterone Acetate.[94]

Testosterone acetate (1.0g, 3.0mM) was dissolved in acetic acid (20ml). CAN (3.32g, 6.0mM) was dissolved in water (2ml) and added to the acetic acid solution. The solution was stirred overnight at room temperature to give a colourless solution. The usual work-up gave a brown syrup which crystallised on cooling to give brown crystals, T.L.C. of whi ch showed the only product was unchanged starting material with degradation products. The product was recrystallised from aqueous acetone to give testosterone acetate, (0.70g, 70%) M.P. 139-40° (lit[94] M.P. 141-2.5°) ν max 2930, 1715, 1660, 1265cm^{-1}, N.M.R. absorptions at τ 4.24, 5.27, 5.38, 5.51, 7.97, 8.80, 9.17.

Δ9(11) Progesterone.[121]

9(11) progesterone (0.1g, 0.32mM) was dissolved in acetic acid (3ml). CAN (0.35g, 0.64mM) was dissolved in water (1ml) and added to the acetic acid solution. The solution was stirred overnight at room temperature to give a colourless solution. The usual work-up gave a brown syrup which crystallised on cooling. The product was recrystallised from acetone/hexane to give Δ9(11) progesterone (0.070g, 70%) M.P. 124-6° (lit[121] M.P. 122-4°) ν max 3000, 1700, 1660, 1620cm^{-1}, N.M.R. absorptions at τ 4.24, 4.45, 7.87, 8.69, 9.39.

The reaction was repeated varying both reaction time and quantity of ceric. Reaction with two mole-equivalents of ceric for fifteen minutes yielded starting material in 90% yield. Reaction with eight mole-equivalent for fifteen minutes and one hour yielded starting material in 90% yield in

each case.

Androst-2-en-17β-yl acetate.[89]

Androst-2-enyl acetate (0.1g, 0.32mM) was dissolved in acetic acid (6ml). CAN (0.35g, 0.63mM) was dissolved in water (1ml) and added to the acetic acid solution. The solution was stirred overnight at room temperature to give a colourless solution. The usual work-up gave a yellow syrup which crystallised on cooling. T.L.C. indicated this consisted of starting material with degradation products. The product was recrystallised from ether to giveandrost-2-enyl acetate (0.080g, 80%) M.P. 104-6° (lit[89] M.P. 98-9°) ν max 2900, 1715, 1256cm^{-1}, N.M.R. absorptions at τ 4.42, 5.34, 5.41, 5.50, 7.99, 9.21, 9.24.

Stigmasta-4,22-dien-3-one.[117]

Stigmastadienone (0.1g, 0.24mM) was dissolved in acetic acid (9ml). CAN (0.27g, 0.49mM) was dissolved in water (2ml) and added to the acetic acid solution. The solution was stirred overnight to give a colourless solution. The usual work-up gave a yellow syrup, which was recrystallised from ethanol to give stigmastadienone (0.08g, 80% M.P. 120-3° (lit[117] M.P. 124.5-5°) ν max 3000, 1660, 1620cm^{-1}, N.M.R. absorptions at τ 4.27, 4.90, 8.81, 9.27.

Dihydrolanosteryl Acetate.[86]

Dihydrolanosteryl acetate (0.1g, 0.21mM) was dissolved in acetic acid (5ml). CAN (0.47g, 0.84mM) was dissolved in water (1ml) and added to the acetic acid solution. The solution was immediately decolourised. The usual work-up gave a brown oil, which was shown by T.L.C. to consist of two major products. Preparative T.L.C. yielded two crystalline compounds.

1. Dihydroagnosteryl acetate (24, 25-dihydrolanosta-7,9(11)dienyl acetate). (0.03g, 30%) M.P. 166-8° (lit[86] M.P. 167-8°) ν max 3450, 2900, 1720,

1675cm^{-1}, N.M.R. absorptions at τ 4.4-4.7 (equivalent to two protons) 5.4-5.6, 7.95, 9.02, 9.13, 9.17.

2. 7-Ketodihydrolanosteryl acetate (0.02g, 20%) M.P. 146-9°(γ from methanol) (lit[86] M.P. 151-2°)νmax 3500, 2920, 1720, 1650cm^{-1}, N.M.R. absorptions at τ 5.4-5.6, 7.95, 9.10, 9.17.

Androstanolone Acetate.[93]

Androstanolone acetate (0.2g, 0.6mM) was dissolved in acetic acid (10ml). CAN (1.33g 2.4mM) was dissolved in water (1ml) and added to the acetic acid solution. The solution was stirred overnight at room temperature to give a colourless solution. The usual work-up gave a brown syrup. T.L.C. indicated extensive degradation had occurred. Preparative T.L.C. enabled the isolation of androstanolone acetate(30mgs, 15%) M.P. 156-8° (lit[93] M.P. 158-9°) confirmed by I.R. and N.M.R. spectra. No other product could be isolated and identified. Acidification and extraction into ether of the bicarbonate washing, gave, after washing with water, drying and evaporation of solvent, a product which was shown by T.L.C. to consist of three or four components. These were judged to be products of ring cleavage with further degradation. No attempt was made to identify the compounds since they were present in very small yield (20mgs, 10% total yield for all four compounds.)

The reaction was repeated with variation in the reaction time and quantity of ceric. Reaction with two mole-equivalents of ceric for four hours yielded starting material in 30% yield. Reaction with four mole-equivalents of ceric for eight hours yielded starting material, androstanolone acetate, in 25% yield.

Non-Steroidal Aromatic Compounds.

Toluene.

Toluene (1g, 10.9mM) was dissolved in acetic acid (100ml). CAN (23.8g, 43.5mM) was dissolved in water (10ml) and added to the acetic acid solution. The solution was stirred overnight at room temperature. The reaction was worked up in the usual manner to give a product which was a mixture of two compounds -

1. Benzyl nitrate, (80%, not isolated pure). ν max 2930, 1635, 1283cm^{-1}, N.M.R. absorptions at τ 4.72.

2. Benzyl acetate, (20%, not isolated pure) ν max 2930, 1720cm^{-1}, N.M.R. absorptions at τ 5.00, 8.08. (The preparation of benzyl nitrate was confirmed by reduction in the usual manner with zinc/acetic acid. This resulted in the disappearance of the N.M.R. peak at τ 4.72, and also of the I.R. nitrate peaks at 1640, 1280, 860cm^{-1}, and the appearance of a peak at τ 5.46, characteristic forbenzyl alcohol.

Benzyl alcohol was acetylated in the usual manner to yield benzyl acetate, which was identical with the ceric oxidation product, thus confirming this product.)

The reaction was repeated using nitric acid as solvent. Toluene (1g, 10.9mM) was added to 3.5N nitric acid (10mls) and stirred vigorously. CAN (23.8g, 43.5mM) was dissolved in 3.5N nitric (30 mls.) The solution was heated on a hot water bath (80°) until colourless (90 minutes). The nitric acid was neutralised by the addition of solid sodium bicarbonate. The volume was increased to 100 mls by the addition of water. The solution was extracted with 3 x 50mls portions of ether. The solution was washed with saturated sodium bicarbonate solution, and water, and dried. Evaporation of the solvent yielded the product, benzaldehyde, (0.8g, 69%) ν max 2950, 1700cm^{-1}. N.M.R. absorptions at τ 0.1, 2-3

(complex aromatic region).

Ethylbenzene.

Ethylbenzene (1g, 9.4mM) was dissolved in acetic acid (90ml). CAN (20.7g, 37.7mM) was dissolved in water (10ml) and added to the acetic acid solution. The solution was stirred overnight at room temperature. The usual work-up yielded a product which was a mixture of two compounds, contaminated with starting material. The two products were:

1. Acetophenone (30%, not isolated pure), ν max 2960, 1680cm^{-1}, N.M.R. absorptions at τ 7.54.

2. α-Phenyl ethyl acetate (70%, not isolated pure) ν max 2960, 1715cm^{-1}, N.M.R. absorptions at τ 3.97, 4.08, 4.19, 4.30 (proton adjacent to acetate), 8.03 (acetate) 8.46, 8.58, (α-methyl group).

The products were identified by comparison with the N.M.R. spectra of authentic samples. α-Phenyl ethyl acetate was prepared by the usual acetylation reaction from α-phenylethanol, prepared by sodium borohydride reduction of acetophenone.

Cumene.[122,123]

Cumene (1g, 8.3mM) was dissolved in acetic acid (35ml). CAN (18.3g, 33.2mM) was dissolved in water (7ml) and added to the acetic acid solution. The solution was stirred at room temperature until colourless (60 hours). The usual work-up yielded a pale brown syrup. T.L.C. showed this consisted of two products in addition to unreacted starting material. The product was chromatographed on silica. Elution with petrolyielded cumene, N.M.R. absorptions at τ 2.80. 6.8-7.5 (maximum height at 7.15, 7 peaks, central proton of isopropyl group, 8.72, 8.83, J = 7Hz, methyl groups of isopropyl group). Elution with chloroform yielded the two products as one fraction. Preparative T.L.C. would not separate the two products, which were shown by N.M.R. and I.R. spectra to be cumyl alcohol (α,α-dimethyl benzyl alcohol) N.M.R. absorptions at τ 2-3 (complex aromatic

region), 8.48 (methyls adjacent tohydroxyl) and 2-phenyl propane-1,2,-diol 1-nitrate, N.M.R. absorptions at τ5.43 (protons adjacent to nitrate), 7.42 (protons attached to C-3 of propane). The N.M.R. spectrum of the crude product showed the reaction product was a mixture of nitrate ester, cumyl alcohol, and cumene in the ratios 2:1:1 respectively.

Reduction of the Above Product

The oxidation products (0.5g) were dissolved in acetic acid (25ml) and zinc (5g) was added. The suspension of zinc was stirred at room temperature for one hour. The zinc was filtered off and the solution poured into ether (100ml) and the ether solution was washed with saturated sodium bicarbonate solution and water, and dried. Evaporation of the solvent yielded the product as a pale brown oil which was shown by T.L.C. to consist of cumyl alcohol and 2-phenyl propane-1,2,-diol. Preparative T.L.C. enabled the isolation of the products; cumyl alcohol (0.1g,10% over complete reaction) M.P. 32-4° (lit[122] M.P. 35-7°) confirmed by N.M.R. spectrum, and 2-phenyl propane-1,2,-diol (0.15g, 11% over complete reaction). This product was recrystallised from aqueous methanol to give white crystals, M.P. 43-4° (lit[123] M.P. 44-5°), N.M.R. absorptions at τ 2-3 (very complex aromatic region), 6.0-6.5 (protons on C-1 and hydroxyls) 7.49 (protons on C-3).

t-Butyl benzene.

t-Butyl benzene (0.5g, 4.0mM) was dissolved in acetic acid (30ml). CAN (4.1g, 8.0mM) was dissolved in water (3ml) and added to the acetic acid solution. The solution was stirred at room temperature for two days. The usual work-up yielded a pale yellow oil which was shown by N.M.R. to be unreacted starting material (0.425g, 85%) N.M.R. absorptions at τ2.6-2.9, 8.70. T.L.C. indicated there were no other products present in the product. Less than 100% return of starting material indicated some degradation had occurred but the products of the degradation could

not be isolated.

Phenylcyclohexane and derivatives.

Phenylcyclohexane.[124,99]

Phenylcyclohexane (1g, 6.7mM) was dissolved in acetic acid (200ml). CAN (13.7g, 26.8mM) was dissolved in water (20ml) and added to the acetic acid solution. The solution was stirred at room temperature until titres showed no ceric remained in the solution (48 hours). The usual work-up yielded the product as a brown syrup (0.9g), I.R. spectrum of which showed the characteristic nitrate peaks at 1640, 1280, 860cm^{-1}.

T.L.C. showed the product consisted in the main of two compounds plus degradation products. The main products were 1-phenylcyclohexan-1-ol and 1-phenylcyclohexane -1,2,diol-2-nitrate (from green T.L.C. spot and reduction to 1-phenyl cyclohexane-1,2-diol). The compounds overlapped on T.L.C. so no attempt was made to separate them. The T.L.C. also had a faint spot for the diol. This was not isolated at this stage.

Reduction of the Above Product

The product (0.85g) was dissolved in acetic acid (10ml) and zinc dust (5g) was added. The suspension was stirred at room temperature for one hour. The zinc was filtered off, and the acetic acid solution poured into ethyl acetate, and washed with saturated sodium bicarbonate solution, and water, and dried. Evaporation of the solvent yielded the product as a colourless oil. T.L.C. showed the product consisted of three compounds. Preparative T.L.C. enabled the separation of the compounds. They were identified as being;

1. phenylcyclohexane (0.1g, 10%) ν max 2950cm^{-1}, N.M.R. absorptions at τ 2.4-2.9 (complex aromatic region, 7.6-8.8 (hump as with steroids);

the other two fractions were recrystallised from petrol to give:

2. phenylcyclohexan-1-ol (0.14g, 13%) M.P. 60-2° (lit[124] M.P. 62-35°)

ν max 3650, 2950cm^{-1}, N.M.R. absorptions at τ 2.35-2.8, (complex aromatic region (8.0-8.5, no peak for hydroxyl group),

3. Phenylcyclohexane-1,2-diol (0.6g, 50%) M.P. 92-4° (lit[99] M.P. 93.5-94.5°) ν max 3630, 2950cm^{-1}, N.M.R. absorptions at τ 2.4-3.0, (complex aromatic region), 7.8-8.8, no peak for hydroxyl groups, and no peak visible for proton adjacent to secondary hydroxyl group.

The product also contained several unidentified compounds which were not isolated. These were present in very low yield. These were possibly further oxidation or cleavage products of phenylcyclohexane-1,2-diol.

1-Phenylcyclohex-1-ene.[124,99]

1-Phenylcyclohex-1-ene (0.515g, 3.5mM) was dissolved in acetic acid (200ml). CAN (3.572g, 7.0mM) was dissolved in water (20ml) and added to the acetic acid. The solution was stirred at room temperature until titres showed no ceric remained in the solution (one hour). The usual work-up gave a pale yellow oil (0.4g) as product.

T.L.C. of this oil showed it consisted of a mixture containing the same compounds as were obtained in the previous oxidation reaction with several products, not previously obtained. The crude product was taken on into the reduction reaction without isolation of the products.

Reduction of the Above Product

The product obtained above (0.35g) was reduced with zinc (4g) in acetic acid (20ml) as described for the phenylcyclohexane oxidation product.

Preparative T.L.C. enabled isolation of the products, which were shown to be 1-phenylcyclohexan-1-ol (0.056g, 10%) M.P. 61-3° (lit[124] M.P. 60°) and 1-phenylcyclohexane-1,2-diol (0.18g, 30%), M.P. 93-4° (lit[99] M.P. 93.5-4.5°). The rest of the product consisted of eight unidentified products, none of which were isolated. All were present in 10% yield or less. The two identified products were confirmed by T.L.C.,

I.R. and N.M.R. comparison with the authentic compounds.

1-Phenylcyclohexan-1-ol.

1-Phenylcyclohexan-1-ol (0.5g, 2.85mM) was dissolved in acetic acid (200ml). CAN (3.11g, 5.7mM) was dissolved in water (20ml) and added to the acetic acid solution. The solution was stirred at room temperature till titres showed no ceric remained in the solution (five hours). The usual work-up gave the product as a pale yellow oil (0.4g). T.L.C. showed the product consisted of a mixture of the same compounds as was obtained in the previous two oxidation reactions, in the approximate ratios of 1-phenylcyclohexanol (20%), 1-phenylcyclohexane-1,2-diol 2-nitrate (40%) and 1-phenylcyclohexane-1,2,-diol (10%). The reaction product was reduced in the usual manner to facilitate isolation of the products.

Reduction of the Above Product.

The product (0.35) was reduced with zinc (4g) in acetic acid (20ml) as described for the previous two reactions. Preparative T.L.C. was again used to isolate the products, which were shown to be 1-phenylcyclohexanol (0.165g, 31%), and 1-phenylcyclohexane-1,2-diol (0.22g, 41%). These compounds were identified by M.P. and T.L.C., I.R. and N.M.R. comparison with the authentic compounds.

Methyl Tetralin.[123,96]

Methyl tetralin (1g, 6.85mM) was dissolved in acetic acid (50ml). CAN (15.98g, 27.4mM) was dissolved in water (10ml) and added to the acetic acid solution. The solution was stirred at room temperature until colourless, (48 hours). The usual work-up yielded a pale brown syrup (1.25g). T.L.C. of this syrup gave only one spot, but N.M.R. and I.R. showed it to consist of two compounds, an acetate (in 60% yield) and a nitrate ester (in 30% yield). Chromatography could not separate these compounds. The syrup had the following spectral characteristics; N.M.R. absorptions at τ 2.2-3.2 (aromatic protons), 3.9-4.2 (protons adjacent to

nitrate), 4.6-4.9 (benzylic protons) 8.0 (acetate), ν max 2920, 1720, 1640, 1280, 1260, 860cm^{-1}. (Formation of the acetate was confirmed by repetition of the reaction in propionic acid, which yielded a product, of similar constitution, but with an N.M.R. spectrum containing the characteristic propionate peaks at τ 7.60, 7.72, 7.86, 7.98, 8.61, 8.88).

The compounds were confirmed as being nitrate and acetate derivatives of methyl tetralin by the mass spectral data which gave a parent ion peak at m/e=204 ($C_{13}H_{16}O_2$ the acetate), and m/e=223($C_{11}H_{13}NO_4$, the nitrate).

The products were finally identified as being 1-methyl 1-hydroxy tetralin acetate, and 1-nitratomethyl 1-hydroxy tetralin by conversion to the corresponding 1-methyl 1-hydroxy tetralin and 1-hydroxymethyl 1-hydroxy tetralin as detailed below.

Reduction of nitrate ester.[123]

A portion (0.5g) of the product from the above reaction was dissolved in acetic acid (10ml) and zinc dust (5g) was added. The reaction was stirred for one hour at room temperature. The zinc was filtered off and washed with acetic acid. The acetic acid solution was poured into ether (100ml) and washed with saturated sodium bicarbonate solution, and water and dried. The solution was evaporated to dryness to yield a brown syrup. T.L.C. of the product showed it consisted of the same acetate as before and a more polar product. Preparative T.L.C. enabled the isolation of this compound. Recrystallisation from ethyl acetate gave white crystals of 1-hydroxymethyl 1-hydroxy tetralin (0.05g,) M.P. 102-4° (lit[123] M.P. 103-4°) N.M.R. absorptions at τ 2.35-3.0 (aromatic). 6.35 (CH_2 of hydroxymethyl), 6.85-7.4 (benzylic protons) 7.7-8.4 (hump as in steroids), ν max 3600, 2950cm^{-1}. This confirmed the nitrate compound as being 1-nitratomethyl 1-hydroxy tetralin.

Hydrolysis of Acetate.[96]

A portion (0.10g) of the product from the previous reaction was

dissolved in aqueous ethanol (10mls of 90% alcohol). Sodium hydroxide (0.1g) was added and the solution was stirred overnight at room temperature. The alcoholic solution was poured into ether (100ml) and washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water, and dried. Removal of the solvent gave the product. T.L.C. showed much degradation had occurred (eg reaction of nitrate ester). T.L.C. enabled the isolation of the major product.

Recrystallisation from benzene gave white crystals of 1-methyl 1-hydroxy tetralin, M.P. 88-9° (lit[96] M.P. 88-9°) ν max 3500, 2930cm^{-1}, N.M.R. absorptions at τ 2.4-2.6, 2.7-3.0, 7.2, 7.26, 7.97, 8.10, 8.16, 8.52 (ie 7.2-8.5).

This confirmed the acetate as being 1-methyl 1-hydroxy tetralin acetate.

Reactions of Oxidation Products of Oestrone Acetate.

Reduction.

1. With Pd/C.

3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (1g, 2.6mM) was dissolved in ethylacetate (50ml) and hydrogenated at atmospheric pressure and room temperature using pre-reduced 10% Pd/C (1g) as catalyst. Initially there was no reaction. After twenty minutes, rapid uptake of hydrogen commenced and continued for thirty minutes. When the rate dropped after consumption of 60mls (calculated volume 58ml), the catalyst was filtered off on Celite and the solvent was evaporated to give a yellow syrup which crystallised on standing. Recrystallisation from acetone or methanol yielded 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (0.51g, 60%) M.P. 198-202° (Found C74.31% H5.26% $C_{20}H_{24}O_5$ requires C69.76% H6.98%) ν max 3580, 2940, 1728, 1220cm^{-1}, N.M.R. absorptions at τ 2.50, 2.66, 2.98, 3.10, 5.62 (complex multiplet) 7.75, 8.90. Comparison with other methods shows this is the expected compound. (Analysis figures are falsified by the presence of carbon from the catalyst.)

2. With Zinc/Acetic Acid.

3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.2g, 0.52mM) was dissolved in acetic acid (10ml) and freshly prepared zinc dust (2g) was added. The mixture was stirred at room temperature for 1½ hours. The zinc was filtered off and washed well with acetic acid. The acetic acid solution was diluted to 100mls with methylene chloride. The methylene chloride solution was washed exhaustively with saturated sodium bicarbonate solution, and water. Drying and evaporation of the solvent yielded the product as a pale yellow syrup which crystallised to white crystals on standing. Recrystallisation from acetone, or methanol,

yielded 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (0.100g, 60%) M.P. 199-202° (Found C69.84% H6.92% $C_{20}H_{24}O_5$ requires C69.76% H6.98%) ν max 3580, 2940, 1728, 1220cm^{-1} N.M.R. absorptions at τ 2.50, 2.66, 2.98, 3.10, 5.6 (complex multiplet) 7.75, 8.90.

3. With Zinc/Ethanol.

3,9α,11β-trihydroxyoestra-1,3,5(10)-trien -17-one 3-acetate 11β-nitrate (0.2g, 0.52mM) was dissolved in 90% ethanol (60mls) and zinc (2g) was added. The solution was refluxed for two hours. The zinc was filtered off, and well washed with ethanol. The volume was reduced to 20mls, and chloroform was added (80ml). The solution was washed with water (3 times), dried and the solvent evaporated to give the product as a pale brown syrup. Recrystallisation from acetone/hexane gave pale brown crystals M.P. 194-200°. Recrystallisation from acetone gave white crystals, (0.11g, 62%) M.P. 198-202°, confirmed by I.R., N.M.R. and T.L.C. to be the diol prepared by the previous two methods.

4. With Raney Nickel.

Raney Nickel was prepared as in Organic Syntheses, 21, 15.

The steroid (0.25g, 0.64mM) was dissolved in dioxan (10ml). Raney nickel(5ml, 3g approximately) in alcohol was added, and the suspension was stirred at 0° for eight hours. The nickel was filtered off on Celite, and the Celite washed twice with ether (2 x 50ml portions). The ether solution was washed twice with water and dried. Evaporation of the solvent yielded the product which was shown by T.L.C. and N.M.R. to consist predominantly of the usual reduction product. Recrystallisation from acetone yielded the product 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (0.194g, 70%) M.P. 196-200°, confirmed by I.R. and N.M.R. spectra, and T.L.C.

The structure of the above compound was confirmed by preparation of the diacetate as detailed below, and by conversion to the known

compound, 3,9α-dihydroxyoestra-1,3,5(10)-triene-11,17-dione 3-acetate by oxidation with Jones reagent (as detailed on P152).

Preparation of 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3,11β-diacetate.

The steroid (0.070g, 0.2mM) was dissolved in pyridine (2ml) and acetic anhydride (1ml, 10mM) was added. The solution was left overnight at room temperature. The usual work-up gave the product as white crystals. The product was recrystallised from acetone to give white crystals of 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3,11β-diacetate (70mgs, 90%) M.P. 202-5° (Found C68.16% H6.74% $C_{22}H_{26}O_6$ requires C68.39% H6.72%) ν max 3550 (small), 2920, 1730 (very broad), 1270, 1220cm^{-1} N.M.R. absorptions at τ 2.65, 2.81, 3.10, 4.32, 7.74, 8.21, 8.99. T.L.C. showed the compound had a polarity corresponding to a free hydroxy group ie. Rf similar to 3,9α-dihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate.

Reaction of Nitrate Ester with Base.

1. With Potassium Acetate.[73]

3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.1g, 0.26mM) was dissolved in dioxan (3ml) and potassium acetate (0.084g, 4 mole equivalents) in methanol (3ml) was added. The solution was warmed under reflux at 40° for one hour. The solution was poured into ether (30ml) and washed with water (5 x 10ml portions). The solution was dried and the solvent evaporated to give the product as a pale yellow syrup which would not crystallise. T.L.C. and N.M.R. indicated the product was 3-hydroxy-9α,11-epoxyoestra-1,3,5(10)-trien-17-one 3-acetate. Preparative T.L.C. enabled the isolation of the product. Recrystallisation from acetone/hexane yielded 3-hydroxy-9α,11-epoxyoestra-1,3,5(10)-trien-17-one 3-acetate (51mgs, 60%) M.P. 160-1° (lit[73] M.P. 160-2°) ν max 2940, 1742cm^{-1} N.M.R. absorptions at τ 2.75, 3.10, 5.90, (complex multiplet) 7.75, 9.10.

2. With Sodium Bicarbonate.[73]

3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.1g, 0.26mM) was dissolved in 90% ethanol (25ml) and added to sodium bicarbonate (0.1g) in 90% ethanol (25ml). The solution was warmed at 40° for ten minutes and poured into chloroform (200ml). The solution was washed five times with water (50ml portions), dried and the solvent evaporated to give a brown syrup which crystallised on standing. Recrystallisation from acetone/hexane gave purple crystals, M.P. 142-52°. Further recrystallisation from acetone/hexane gave white crystals of 3-hydroxy-9α,11-epoxyoestra-1,3,5(10)-trien-17-one 3-acetate (40mgs, 48%) M.P. 158-60° (lit[73] M.P. 160-2°) ν max 2930, 1740, 1210cm^{-1} N.M.R. absorptions at τ 2.75, 3.10, 5.90, 7.75, 9.10.

3. With Sodium Carbonate.[101]

3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.2g, 0.52mM) was dissolved in 90% ethanol (50ml) and sodium carbonate (0.2g) was added as solid. The suspension was stirred at 60° for one hour during which time the solution turned a deep red colour. The solution was cooled to room temperature, neutralised by the addition of acetic acid / filtered and the volume reduced to 10ml, keeping the temperature below 60°. Chloroform (90ml) was added and the solution was washed with water (5 x 20ml portions), dried and evaporated to dryness. The product, a dark blue syrup, was recrystallised twice from ether to give 3-hydroxy-9β-oestra-1,3,5(10)-trien-11,17-dione (56mgs, 40%) M.P. 208-10° (lit[101] M.P. 204-7°) ν max 3550, 2920, 1730, 1695, 1250cm^{-1} N.M.R. absorptions at τ 3.16, 3.35, 6.3 (complex multiplet, 9β-hydrogen), 9.08.

4. With Sodium Hydroxide.[101]

3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.1g, 0.26mM) was dissolved in methanol (10ml) and sodium hydroxide (0.075g) was added. The solution was refluxed for one hour, cooled

and neutralised by the addition of acetic acid. The solution was poured into chloroform (70ml), washed tillneutral with saturated sodium bicarbonate solution, and water, and dried. Evaporation of the solvent and recrystallisation from ether gave 3-hydroxy-9β-oestra-1,3,5(10)-trien-11,17-dione (30mgs, 20%) M.P. 205-10° (lit[101] M.P. 204-7°) I.R. and N.M.R. spectra showed the compound to be identical with that prepared above.

Reduction with Sodium Borohydride.[73]

3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.25g, 0.65mM) was refluxed for one hour in 80% ethanol (6ml) containing sodium borohydride (0.325g, 8.6mM). The excess borohydride was destroyed with dilute hydrochloric acid, and the solution was poured into chloroform (50ml). The chloroform solution was washed till neutral with saturated sodium bicarbonate, and water. The solution was dried, and the solvent evaporated to give the product as white crystals. Recrystallisation from acetone gave 3,11α,17β-trihydroxy-9βoestra-1,3,5(10)-triene(0.1g, 52.) M.P. 258-260° (lit[73] M.P. 254-5°) ν_{max} 3550, 2920. The compound was too insoluble in chloroform to allow the N.M.R. spectrum to be obtained.

Reduction with Lithium Aluminium Hydride.[73]

3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (0.1g, 0.26mM) was added slowly and the solution was refluxed for one hour. Ethyl acetate was added to destroy excess lithium aluminium hydride, and the solution was washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water. The solution was dried and the solvent evaporated to give the product as a colourless syrup which crystallised on standing. Recrystallisation from acetone gave 3,11α,17β-trihydroxy-9β-oestra-1,3,5(10)-triene(40mgs,53%) M.P. 259-61° (lit[73] M.P. 254-5°) I.R. and T.L.C. confirmed the product was identical with the product prepared above.

Reduction of 3,9α,11β,17β-tetrahydroxyoestra-1,3,5(10)-triene3,17βdiacetate 11β-nitrate.

The steroid (0.2g,0.46mM) was dissolved in acetic acid (20ml) and freshly prepared zinc dust (2g) was added. The solution was stirred at room temperature for 90 minutes. The solution was filtered and the zinc was well washed with acetic acid (2 x 10ml portions). Methylene chloride (100ml) was added and the solution was washed with saturated sodium bicarbonate, till neutral, and water. Drying and removal of solvent gave the product as a colourless syrup which crystallised on cooling. Recrystallisation from methanol gave 3,9α,11β,17β-tetrahydroxyoestra-1,3,5(10)-trien 3,17β-diacetate (0.11g 60%) M.P. 156-7° (Found C68.04%, H7.35% $C_{22}H_{28}O_6$ requires C68.02%,H7.22%) ν_{max} 3620, 2930, 1750, 1730, 1250, 1205cm^{-1} N.M.R. absorptions at τ 2.58, 2.66, 3.04, 5.2-5.4 ($C_{17}H$) 5.72 ($C_{11}H$) 7.76, 8.00, 9.00.

Reductionof 3,9β,11β,17β-tetrahydroxyoestra-1,3,5(10)-triene-3,17β-diacetate 11β-nitrate.

The steroid (0.04g,0.046mM) was dissolved in acetic acid (4ml) and freshly prepared zinc dust (0.4g) was added. The solution was stirred at room temperature for 90 minutes. The solution was filtered, and the zinc was washed well with acetic acid (2 x 2ml portions). Methylene chloride (50ml) was added and the solution was washed with saturated sodium bicarbonate solution and water. Drying and removal of solvent gave the product as a colourless syrup which crystallised on cooling. Recrystallisation from methanol gave 3,9β,11β,17β-tetrahydroxyoestra-1,3,5(10)-triene 3,17β-diacetate (0.02g 55%) M.P. 164-5° (Found C67.89%, H7.21. $C_{22}H_{28}O_6$ requires C68.04%,H.7.22%) ν max 3620, 2930, 1750, 1730 1250, 1205cm^{-1}, N.M.R. absorptions at τ 2.52, 2.62, 3.06, 3.12, 5.3-5.45, 5.62 ($C_{11}H$) 7.72, 8.01, 8.84.

Alternative method of separation of the two isomeric oxidation products.

The crude product from the CAN oxidation of oestradiol diacetate (0.5g) was dissolved in acetic acid (20mls) and freshly prepared zinc dust (5g) was added. The solution was stirred at room temperature for ninety minutes. The zinc was filtered off, and well washed with acetic acid (2 x 10ml portions). Methylene chloride was added (100ml) and the solution was washed with saturated sodium bicarbonate solution and water. Drying and removal of solvent gave the product as a pale yellow syrup. Preparative T.L.C. was used to separate the initial starting material from the reduction product. The N.M.R. spectrum and T.L.C. showed the product consisted of two compounds; 3,9α,11β,17β-tetrahydroxyoestra-1,3,5(10)-trien-17-one 3,17β-diacetate and the 9β-isomer. The mixture of compounds was dissolved in acetone, (5ml) and magnesium sulphate (1g) was added. The acetone was boiled off. This was done twice more. The magnesium sulphate was filtered off and washed well with ether. The ether solution was dried using magnesium sulphate. The solution was filtered, and the solvent removed. Preparative T.L.C. now enabled the separation of two compounds of greatly differing polarity. The product of high polarity, low Rf, was recrystallised from methanol to give pure 3,9α,11β,17β-tetrahydroxyoestra-1,3,5(10)-triene3,17β-diacetate (0.103g) M.P. 156-7° confirmed by I.R. and N.M.R. spectra. The other product was shown by N.M.R. to be the acetonide of the 9β compound; 3,9β,11β,17β-tetrahydroxyoestra-1,3,5(10)-triene3,17β-diacetate.

N.M.R. absorptions at τ 2.49, 2.58, 3.04, 3.16, 5.31, 5.43, 5.52, 5.58, 7.75, 8.00. 8.48, 8.54 (Methyls of acetonide separated by different proximities to the aromatic ring), 8.86. The compound would not crystallise and was characterised on spectral properties only.

Attempted Conversion of Acetonide to Free Diol.

The acetonide (approximately 20mgs) prepared above was dissolved in

90% acetic acid (2mls) and left overnight at room temperature. The solution was poured into ether (20ml) and washed with saturated sodium bicarbonate solution and water and dried. Removal of the solvent gave the product which was shown by N.M.R. to consist of unchanged acetonide. The same material was also treated with 90% aqueous acetic acid through which hydrogen chloride gas was bubbled, and ethanol containing 10% anhydrous hydrogen chloride. In both cases unchanged acetonide was the only product isolated.

Reactions of 3,9α,11β-trihydroxyoestra-1,3,5(10)-triene-17-one 3-acetate.

1. With Sodium Hydroxide.[101]

The steroid (0.1g,0.29mM) was dissolved in methanol (10ml) and sodium hydroxide (0.1g) in methanol (10ml) was added. The solution was refluxed for thirty minutes, and then neutralised with excess acetic acid, poured into chloroform, washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water. The solution was dried and the solvent evaporated to give the product as a syrup, which would not crystallise. T.L.C. indicated much degradation had occurred, and preparative T.L.C. enabled the isolation of the main product, which was recrystallised from ether to give 3-hydroxy-9β-oestra-1,3,5(10)-triene-11,17-dione (24mgs, 25%) M.P. 204-8° (lit[101] M.P. 204-7°) ν max 3550, 2920, 1730, 1695cm^{-1}.

With Sodium Carbonate.[101]

The steroid (0.2g,0.58mM) was dissolved in 70% ethanol (100ml) containing sodium carbonate (0.4g). The solution was heated at 50° for thirty minutes, evaporated to 20ml at low temperature, poured into ether (100ml) washed with water, dried and the solvent evaporated to yield the product which was recrystallised from acetone to give 3-hydroxyoestra-1,3,6(10)-trien-11,17-dione (110mgs, 70%) M.P. 200-203° (lit[101] M.P. 199-203°) υ max 3550, 2920, 1740, 1700cm^{-1} N.M.R. absorptions at τ 6.4, 9.18.

With Nitric Acid/Acetic Anhydride.

The steroid (0.1g, 0.29mM) was added, in about 20 portions, to acetic anhydride (5ml). For each addition of steroid, concentrated nitric acid (0.1ml) was also added. There was a vigorous reaction between the acetic anhydride and the nitric acid. After the last addition, the solution was left standing for five minutes. Excess water (15ml) was added and the solution stirred for five minutes at 50°C. The solution was poured into chloroform (50ml) washed with saturated sodium bicarbonate solution, and water. Drying and removal of solvent yielded the product. T.L.C. indicated the presence of several components. Preparative T.L.C. gave the following compounds.

1. 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate 11β-nitrate (57mgs, 50%) M.P. 190-2° (from acetone).

2. 3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3,11β-diacetate (6mgs, 5%) M.P. 202-5° (from acetone). A third compound was unreacted starting material (10mgs, 10%) confirmed by I.R. and N.M.R. spectra.

Reduction with Sodium Borohydride.[73]

The steroid (0.14g,0.39mM) was dissolved in 85% ethanol (12ml) and sodium borohydride (0.20g, 5.3mM) was added. The solution was refluxed for one hour. Excess borohydride was destroyed by the addition of dilute hydrochloric acid. The solution was poured into chloroform (50ml) and washed with saturated sodium bicarbonate solution, and water. The solution was dried and the solvent evaporated to give white crystals, which were recrystallised from acetone to give 3,11β,17β-trihydroxyoestra-1,3,5(10)-triene(50mgs, 42%) M.P. 290-3° (lit[73] M.P. 289-91°) ν_{max} 3550, 2920cm^{-1}. The compound was too insoluble in chloroform to allow the N.M.R. spectrum to be obtained.

Oxidation with Jones Reagent.[64,125]

(Preparation of 3,9α-hydroxyoestra-1,3,5(10)-triene-11,17-dione.

3-acetate).

3,9α,11β-trihydroxyoestra-1,3,5(10)-trien-17-one 3-acetate (0.7g, 2.0mM) in acetone (30ml) at 5°C was treated dropwise with 8N chromic acid (1.0ml) with stirring. The solution was stirred for five minutes, then methanol (1ml) and water (30ml) were added. The steroid was extracted into ethyl acetate. The solution was washed with saturated sodium bicarbonate solution, and water, and dried. The solvent was evaporated to give a gum which was recrystallised from methanol to give 3,9α-dihydroxyoestra-1,3,5(10)triene-11,17-dione 3-acetate (0.25g, 36%) M.P. 247-8° (lit[125] M.P. 235-43°) ν max 3540, 2930, 1750-1700cm^{-1} N.M.R. absorptions at τ 2.53, 2.68, 2.73, 3.00, 3.12, 7.73, 9.14, ν max (C Cl_4) 3600, no peak at 3540cm^{-1} showing no hydrogen bonding of the hydroxyl group.

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Note on Publication.

The following paper has been published and a copy is enclosed.

Oxidation of Ring A-Aromatic Steroids to 9,11β-Diol 11-Nitrates with Ceric Ammonium Nitrate.

By P. J. Sykes, F. J. Rutherford, S. B. Laing, G. H. Phillips and J. P. Turnbull.

A P P E N D I X

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OXIDATION OF RING A-AROMATIC STEROIDS TO 9,11β-DIOL 11-NITRATES WITH CERIC AMMONIUM NITRATE

by P.J. Sykes and F.J. Rutherford,
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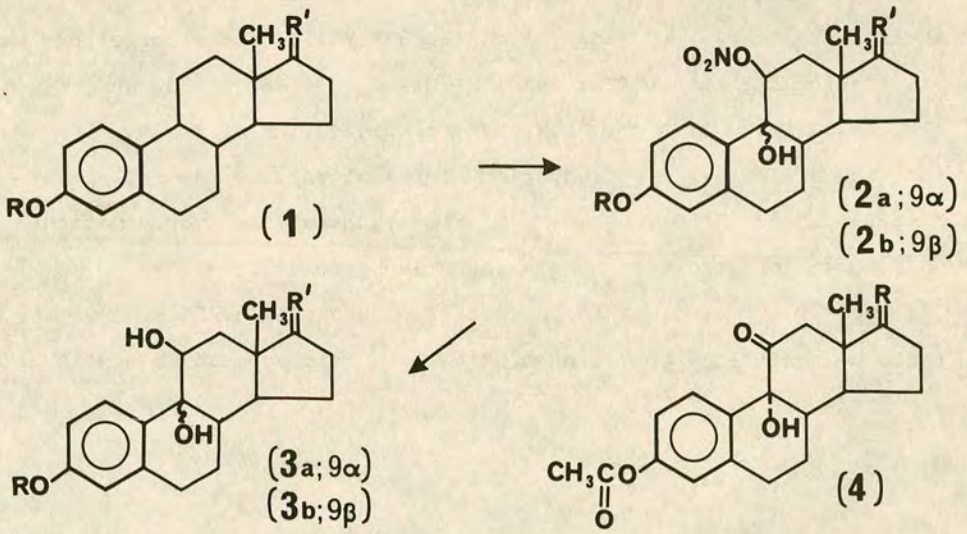
Recently we explored a synthetic route to steroidal oestrogens,[1] the critical stage of which was oxidation by ceric ammonium nitrate of an aromatic 1-methyl group to a 1-formyl group. We now report that in the absence of a 1-substituent oxidation occurs in ring C. Thus, oestrone acetate (1; R=Ac, R'=O) was oxidised in 40 minutes at room temperature by four molar equivalents of ceric ammonium nitrate in aqueous 90% acetic acid to give a readily isolated 69% yield of the corresponding 9α,11β-diol 11-nitrate (2a; R=Ac, R'=O), $\nu_{max.}$ ($CHBr_3$) 1630 and 1270 ($-ONO_2$) and 3570 $cm.^{-1}$(OH). The configuration of the 9-substituent follows from reduction of the nitrate to the 9α,11β-diol (3a; R=Ac, R'=O), by means of zinc or catalytic hydrogenation, and subsequent oxidation to the known 9α-hydroxy-11-ketone (4; R=O)[2] with chromic acid in acetone. The diol (3a; R=Ac,R'=O) could be renitrated to (2a;R=Ac, R'=O) with nitric acid in acetic anhydride.

The configuration of the 11-substituent can be deduced from the nuclear magnetic resonance spectrum (in $CDCl_3$) of the 9α,11β-diol (3a;R=Ac, R'=O), which shows a diffuse apparent triplet (in reality a double double doublet) at τ 5.62 (C<u>H</u>OH, J = <u>ca</u>.2.5 Hz) indicating that the 11-proton is equatorial (α). Further, the signal for the protons of the angular methyl group at τ 8.90, in better agreement with the calculated [3] value (8.82) for the introduction of 9α-hydroxyl (-0.03) and 11β-hydroxyl (-0.25) substituents into oestrone acetate (9.10) than that for the alternative 9α,11α-diol (9.04). The nitrate (2a;R=Ac, R'=O) also exhibits a diffuse triplet (a double doublet) for the equatorial 11α-proton at τ 4.17 (J = <u>ca</u>.3 Hz),the downfield shift relative to the parent alcohol indicating that the 11-hydroxyl carries the nitrate ester. Further, the signal for the angular methyl protons at τ 8.97 is shifted downfield relative to that of oestrone acetate.



In the oxidation of oestrone acetate only a small quantity (ca.5%) of an isomeric diol nitrate was formed. The oxidation of materials with different 17-substituents was less stereospecific; for example, oestradiol diacetate (1;R=Ac, R'=H, β-OAc) gave 46% of one diol nitrate and 17% of a second. The predominant 9α,11β-isomer (2a;R=Ac, R'=H, β-OAc) showed signals at τ 9.06 (13-CH_3) and 4.26 (multiplet, $CHONO_2$) and on reduction gave the 9α,11β-diol (3a;R=Ac, R'=H, β-OAc), τ 8.95 (calc.from oestradiol diacetate, 8.90) and 5.57 (apparent triplet, J=3 Hz). Oxidation of the diol with chromic acid gave the ketol (4; R=H, β-OAc), which did not show hydrogen bonding in dilute solution in carbon tetrachloride, $\nu_{max.}$ 3600 (OH), further confirming that the 9-hydroxyl is in the α-configuration.[2]

The minor isomer is probably the 9β,11β-diol 11-nitrate (2b; R=Ac, R'=H, β-OAc), for the 11-proton is again equatorial in the nitrate (τ 3.93, J = ca. 3 Hz) and in the diol (3b; R=Ac, R' = H, β-OAc) (τ 5.41, J = ca. 3 Hz) formed from it on reduction. The 13-methyl protons in the nitrate appear at τ 8.95, downfield compared with the 9α-isomer, and on reduction to the diol the same downfield shift (-0.11) to τ 8.84 was observed as in the case of the 9α-isomers.

Oestrone methyl ether (1; R=CH_3, R'=O) reacted faster than oestrone acetate to similarly give a mixture of 9α,11β- and 9β,11β-diol 11-nitrates; reaction for a longer time with an excess of oxidant gave a more complex mixture. The effect on the oxidation of other changes in the 3- and 17-substituents is under investigation.

The oxidation of oestrone acetate is envisaged as proceeding first by oxidation at the benzylic 9-hydrogen, to give 9(11)-dehydro-oestrone acetate directly or via dehydration of a 9-hydroxy intermediate. This is supported by the observation that 9β-oestrone

acetate, 9α-hydroxy-oestrone acetate and 9,11-dehydro-oestrone acetate all give the same product (2a; R=Ac, R'=O) with ceric ammonium nitrate, in yields of 44%, 30% and 31% respectively. Further, oxidation does not occur through 9α,11α-epoxy-oestrone acetate or 11-oxo-oestrone acetate for these are not similarly oxidised. We postulate formation of an α-face complex with a ceric ion which allows nucleophilic attack by nitrate anion at the 11β-position to give a free radical at the 9-position which is further oxidised by ceric ion to yield a carbonium ion which then reacts with a water molecule to yield a 9α- or 9β-hydroxyl.

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